Exploring the link between autoimmune disorders and the risk of developing multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system which leads to demyelination and neurodegeneration. While the cause of MS remains unknown, current research points to key genetic, environmental, and infectious factors which play a role in the onset of disease. The aim of the research undertaken in this thesis was to investigate the possible role of autoimmune disorders (AiDs) in the etiology of MS and to determine whether specific AiDs confer an increased risk for MS. The AiDs examined in this thesis are rheumatoid arthritis (RA), type-1 diabetes (T1D), psoriasis, Crohn's disease (CD), ulcerative colitis (UC), systemic lupus erythematosus (SLE), celiac disease, hypothyroidism, and hyperthyroidism. Published studies yielded conflicting results; some studies found that T1D, psoriasis, CD, SLE, and hypothyroidism were associated with an increased risk of MS, while others found no evidence of an association with MS.

The association between AiDs and the risk of MS was studied using data from the Canadian, Italian, and Norwegian components of the Environmental Risk Factors in Multiple Sclerosis (EnvIMS) study, a multi-national case-control study. Cases (N = 2,242) were frequency matched to controls (N = 3,992) on sex and age in each country. Three exposure windows were defined to assess the association between the AiDs and MS; exposure window one (EW1) was the diagnosis of the AiD any time prior to MS, exposure window two (EW2) required a minimum 5-year time lag between the diagnosis of the AiD and MS, and exposure window 3 (EW3) only included AiDs diagnosed at age 18 years or younger. The association between the AiDs and MS in each exposure window was explored in two ways: 1) the association between having *any* AiD and the risk of MS, and 2) the association between each of the AiDs and the risk of MS (for EW1 and EW2 only), where numbers were sufficient to permit such analyses. The

statistical approach was logistic regression, adjusted for age and sex, followed by models adjusted for additional confounders.

Our results, presented as adjusted odds ratios (95% CI), suggest evidence of an association between the diagnosis of any AiD and the risk of MS in Canada using EW1 and EW2 (1.47 (1.07-2.03) and 1.61 (1.13-2.29), respectively) and in Italy (1.36 (1.02-1.82) and 1.41 (1.03-1.93), respectively) adjusted for age and sex. This association was not evident when the exposure period was defined as EW3 in Canada (0.95 (0.53-1.73)) or in Italy (1.26 (0.76-2.07)). An increased risk of MS related to the presence of any AiD was not observed in Norway using EW1 (1.00 (0.77-1.30), EW2 (1.09 (0.83-1.45)), or EW3 (0.75 (0.48-1.18)). When AiDs were examined individually, hypothyroidism was found to be associated with an increased risk of MS. Specifically in Canada when the exposure period was defined as EW1 or EW2 (1.92 (1.14-3.23) and 2.24 (1.25-4.01), respectively) and in Italy using exposure period EW1 (1.93 (1.12-3.32)) when adjusting for age, sex, and past body size. This increased risk of MS was not observed in Norway using EW1 or EW2 (1.13 (0.68-1.88) and 1.19 (0.66-2.15), respectively). Psoriasis also showed an increased risk of MS in Canada (1.86 (1.03-3.37)), but not in Italy (1.38 (0.77-2.47)) or Norway (1.31 (0.89-1.93)), when using EW1 after adjusting for age, smoking, smoking history, and past body size.

Our findings suggest that having *any* AiD may increase the risk of MS when the exposure window is not restricted to the childhood or adolescent period. We also found that hypothyroidism showed the strongest association with an increased risk MS when the exposure window is defined as any time prior to MS in both Canada and Italy and with a 5-year time lag prior to MS in Canada. These findings could indicate there is a common genetic or environmental risk factor linking hypothyroidism and MS.

Résumé

La sclérose en plaques (SP) est une maladie inflammatoire chronique du système nerveux central qui engendre de la démyélinisation et neurodégénérescence. Bien que la cause de la SP demeure inconnue, de récentes recherches mettent en évidence des facteurs génétiques, environnementaux et infectieux qui jouent un rôle important dans le développement de cette maladie. Le but de la recherche entreprise dans cette thèse était d'investiguer le rôle des maladies auto-immunes (MAIs) dans l'étiologie de la SP et de déterminer si certaines MAIs confèrent un risque accru de SP. Les MAIs examinées dans cette thèse sont la polyarthrite rhumatoïde (PR), le diabète de type 1 (DT1), le psoriasis, la maladie de Crohn (MC), la colite ulcéreuse (CU), le lupus érythémateux disséminé (LED), la maladie cœliaque, l'hypothyroïdie et l'hyperthyroïdie. Les études existantes sur ce sujet ont fourni des résultats contradictoires ; certaines études ont démontré que le DT1, le psoriasis, la MC, le LED, et l'hypothyroïdie étaient associés à un risque accru de SP, tandis que d'autres n'ont trouvé aucune association avec la SP.

La relation entre les MAIs et le risque de SP a été étudiée en utilisant des données des composantes canadienne, italienne, et norvégienne de l'étude « Environmental Risk Factors in Multiple Sclerosis » (EnvIMS), une étude de cas multinationale. Les cas (N = 2 242) ont été appariés pour la fréquence à des contrôles (N = 3 992) en fonction du sexe et de l'âge dans chaque pays. Trois périodes d'exposition ont été définies pour évaluer l'association entre les MAIs et la SP; la première période d'exposition (EW1) correspondait au diagnostic de la MAI à n'importe quel moment avant la SP, la deuxième période d'exposition (EW2) nécessitait un temps minimum de 5 ans entre le diagnostic de la MAI et la SP, et la troisième période d'exposition (EW3) incluait seulement les MAIs diagnostiquées à l'âge de 18 ans ou moins. La relation entre les MAIs et la SP dans chaque période d'exposition a été investiguée de deux

façons : 1) l'association entre avoir au moins une MAI et le risque de SP, et 2) l'association entre chacune des MAIs et le risque de SP (seulement pour EW1 et EW2) quand le nombre de personnes affectées était suffisant pour permettre de telles analyses. Nous avons utilisé des régressions logistiques, ajustées en fonction de l'âge et du sexe, suivies de modèles ajustés pour des facteurs de confusion supplémentaires.

Nos résultats, présentés en rapports de cotes ajustés (95% CI), suggèrent une association entre le diagnostic d'au moins une MAI et le risque de SP au Canada en considérant EW1 et EW2 (1.47 (1.07-2.03) et 1.61 (1.13-2.29), respectivement) et en Italie (1.36 (1.02-1.82) et 1.41 (1.03-1.93), respectivement) en ajustant pour l'âge et le sexe. Cette association n'a pas été observée quand la période d'exposition était définie comme EW3 au Canada (0.95 (0.53-1.73)) et en Italie (1.26 (0.76-2.07)). Un risque accru de SP lié à avoir une MAI n'a pas été observé en Norvège en considérant les périodes d'exposition EW1 (1.00 (0.77-1.30), EW2 (1.09 (0.83-1.45)), et EW3 (0.75 (0.48-1.18)). Lorsque les MAIs ont été examinées individuellement, l'hypothyroïdie s'est avérée associée à un risque élevé de SP. Plus précisément au Canada, lorsque la période d'exposition a été définie comme EW1 ou EW2 (1.92 (1.14-3.23) et 2.24 (1.25-4.01), respectivement), et en Italie en considérant la période d'exposition EW1 (1.93 (1.12-3.32)) en ajustant pour l'âge, le sexe, et l'historique de taille corporelle. Ce risque augmenté de SP n'a pas été observé en Norvège en considérant EW1 ni EW2 (1.13 (0.68-1.88) et 1.19 (0.66-2.15), respectivement). Le psoriasis a également été associé à un risque accru de SP au Canada (1.86 (1.03-3.37)), mais pas en Italie (1.38 (0.77-2.47)) ni en Norvège (1.31 (0.89-1.93)), en considérant EW1 après ajustement en fonction de l'âge, le tabagisme de seconde main, les antécédents de tabagisme, et l'historique de taille corporelle.

Nos résultats suggèrent qu'avoir une MAI peut augmenter le risque de SP lorsque la période d'exposition n'est pas limitée à la période de l'enfance ou de l'adolescence. Nous avons également démontré que l'hypothyroïdie présentait un fort lien avec un risque accru de SP lorsque la période d'exposition est définie comme n'importe quel temps précédant la SP au Canada et en Italie et avec un minimum de 5 ans avant la SP au Canada. Ces résultats pourraient indiquer qu'il existe certains facteurs de risque génétiques ou environnementaux communs entre l'hypothyroïdie et la SP.

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Contribution of authors

EnvIMS Study Design

The EnvIMS study was collaboratively designed by Dr. Christina Wolfson, Dr. Maura Pugliatti, Dr. Trond Riise, and Dr. Kjell-Morten Myhr, as well as researchers from Canada, Italy, Norway, Sweden, and Serbia. Data from the EnvIMS study were used in this thesis to explore the relationship between autoimmune disorders and the risk of MS.

Thesis Analysis Plan and Statistical Analysis

Mégane Bouchard created an analysis plan which was discussed and revised with Dr. Christina Wolfson and Dr. Erica Moodie. The statistical analysis was carried out under the supervision of Dr. Erica Moodie, and results were carefully reviewed by both Dr. Christina Wolfson and Dr. Erica Moodie.

Thesis Writing, Editing, Reviewing

All chapters of this thesis, including the manuscript “Exploring the link between autoimmune disorders and the risk of developing multiple sclerosis: An EnvIMS study”, were written by Mégane Bouchard. All sections of this thesis and the manuscript were reviewed and edited by Dr. Christina Wolfson, Dr. Maura Pugliatti, and Dr. Erica Moodie. The manuscript was also reviewed by Dr. Trond Riise and Dr. Kjell-Morten Myhr.

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List of Abbreviations

MS = multiple sclerosis

CNS = central nervous system

RRMS = relapsing-remitting multiple sclerosis

SPMS = secondary progressive multiple sclerosis

PPMS = primary progressive multiple sclerosis

PRMS = progressive-relapsing multiple sclerosis

EBV = Epstein-Barr virus

AiD(s) = autoimmune disease(s)

RA = rheumatoid arthritis

T1D = type-1 diabetes

CD = Crohn's disease

UC = ulcerative colitis

SLE = systemic lupus erythematosus

EnvIMS = Environmental Risk Factors in Multiple Sclerosis

PwMS = persons with MS

MRI = magnetic resonance imaging

HLA = human leukocyte antigen

WHO = World Health Organization

IM = infectious mononucleosis

UV = ultraviolet

25[OH]D = 25-hydroxyvitamin D

OR = odds ratio

RR = relative risk

IRR = incidence rate ratio

HR = hazard ratio

KPNC = Kaiser Permanente Northern California

RDD = random digit dialing

ICD = international classification of diseases

IBD = inflammatory bowel disease

EnvIMS-Q = EnvIMS Questionnaire

BMI = body mass index

EW1 = exposure window one (no time lag)

EW2 = exposure window two (five-year time lag)

EW3 = exposure window three (< 18 years old)

95% CI = 95% confidence interval

PMM = predictive mean matching

NMO = Neuromyelitis Optica

Chapter 1: Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) which leads to demyelination and often to progressive neurological deterioration (1). The four major types of disease course of MS are relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and progressive-relapsing MS (PRMS) (1). A high degree of familial clustering of MS has demonstrated that genetics play an important role in the etiology of MS (2). The age of onset of MS peaks around the late 20s and early 30s; Onset in pediatric populations makes up approximately 2-10% of cases and onset past the age of 50 years is rare (1, 2). It is estimated that in 2020, over 2.8 million individuals worldwide had MS (3). The prevalence of MS varies by geographic location; the estimates of MS prevalence in 2020 in Europe and the Americas are 133 and 112 cases per 100,000 people, respectively (3). These estimates are highly variable by region and will be further discussed in Chapter 2. The lifetime risk of MS is lower in Hispanic, Black, and Asian populations and is highest amongst white non-Hispanic populations (2). Women are more commonly affected than men, with a female-to-male incidence ratio varying from 1.5:1 to 2.5:1 (4). A recent Canadian longitudinal study showed recent estimates of the incidence rate ratio for MS in 2013 to be 1.98 (female/male) (5).

The causes of MS are largely unknown, although several genetic, environmental, and lifestyle risk factors have been linked to increase the risk of MS (4). Some of these key environmental factors include Epstein-Barr Virus (EBV) infection, vitamin D deficiency through limited sun exposure and diet, cigarette smoking, and obesity (4, 6, 7). These risk factors are believed to interact with genetics to explain the global variability in the prevalence of MS (8). One interesting association that has recently garnered more attention amongst MS researchers is

the link between having an autoimmune disease (AiD) and the subsequent risk of MS (9). Since MS itself is thought to be an autoimmune disorder, it is important to better understand the relationship between AiDs and subsequent MS since an association between them could point to a shared genetic susceptibility and/or common environmental trigger or immune alterations caused by an AiD which can increase the likelihood of triggering MS. Exploring the link between the presence of specific AiDs and the subsequent risk of MS is the initial step in understanding a possible relationship between the disorders. This thesis will assess the association between nine AiDs and the risk of MS: rheumatoid arthritis (RA), type-1 diabetes (T1D), psoriasis, Crohn's disease (CD), ulcerative colitis (UC), celiac disease, systemic lupus erythematosus (SLE), hypothyroidism, and hyperthyroidism. This association will be explored using data from the Environmental Risk Factors in Multiple Sclerosis (EnvIMS) study; these nine AiDs were selected since they were the ones participants in EnvIMS were asked to report on. When reviewing the published literature, nine studies were identified to have previously examined the relationship between having at least one of these nine AiDs and the risk of MS. The results of these studies across different populations are conflicting and will be discussed in Chapter 2 (10-18). Several of these published studies suffer from limitations, including small sample sizes, inadequate adjustment for confounding, lack of interviewer blinding, and a limited inclusion criteria of MS (i.e., only including individuals with PPMS). The aims of the research presented in this thesis are (a) to summarize the current knowledge on the relationship between having an AiD and the subsequent risk of MS and (b) to explore the relationship between having one of these nine AiDs and the subsequent risk of MS using data from the EnvIMS study in Canada, Italy, and Norway.

Chapter 2 presents selected epidemiological and clinical features of MS and details several risk factors for MS and describes the published literature on the association between AiDs and MS. Chapter 3 introduces the EnvIMS study, the source of data for this thesis. Chapter 4 contains an original manuscript entitled “Exploring the link between autoimmune disorders and the subsequent risk of developing multiple sclerosis: An EnvIMS study” which describes the results of analyses to explore the relationship between the nine AiDs and the subsequent risk of MS. Chapter 5 presents a comprehensive discussion of the research findings and the conclusion. References throughout this thesis will be cited continuously and the reference section will appear after Chapter 5. References for the manuscript will be cited separately to the rest of the thesis and will appear at the end of the manuscript in Chapter 4.

Chapter 2: The Epidemiology of Multiple Sclerosis

This chapter provides an overview of the clinical and pathophysiological features of MS. This is then followed by a description of the various risk factors of MS, including genetics, geography, EBV, vitamin D deficiency, cigarette smoking, and obesity. This chapter concludes with a review of the existing literature on AiDs as risk factors for MS.

2.1 Disease Definition and Clinical Characteristics

MS is considered the most common cause of non-traumatic neurological disability in young adults (19). Different subcategories of MS are characterized by course of disease. RRMS is the most common form of MS, affecting approximately 85% of persons with MS (PwMS) (20). In RRMS, individuals experience relapses, or attacks, of symptoms followed by periods of remission (21). Approximately 35-50% of cases of RRMS will progress to SPMS where individuals experience progressive neurological deterioration with or without relapses and without periods of remission (21). On average, 10-15% of MS cases will begin as PPMS, a disease course characterized by a steady progressive neurological deterioration from disease onset without episodes of remission (20, 21). The fourth subtype of MS, PRMS, is a rarer form of MS which will affect fewer than 5% of PwMS and is characterized as progressive neurodegeneration from disease onset with occasional worsening flare-ups and no periods of remission (20).

MS is widely believed to be an autoimmune disorder mediated by autoreactive lymphocytes, through a mechanism involving CD4+ proinflammatory T cells, which cross the blood-brain barrier, enter the CNS, and cause inflammation resulting in demyelination, gliotic scarring, and axonal loss (22, 23). The resulting lesions usually occur in the white matter of the brain and spinal cord, however, gray matter and cortical lesions are also common (24). The

relapsing-remitting disease course is marked by demyelination and a degree of axonal loss and reactive gliosis, while the progressive disease course is marked by diffuse grey and white matter atrophy and low-grade inflammation and microglial activation at plaque borders (22). The neurological symptoms of MS vary from person to person and can include sensory loss, visual disturbances, optic neuritis, muscle weakness, ataxia, and impaired balance (22). Common physical manifestations of disability caused by MS include leg spasticity, pain, fatigue, cognitive impairment, bladder issues, gait dysfunction, and mood dysregulation (24). Collectively, symptoms of MS lead to a decreased quality of life and individuals with MS are expected to have a shortened life expectancy of between 7-14 years (25). MS is considered an unpredictable, although not fatal, condition (22).

2.2 Diagnostic Criteria

Several MS diagnostic criteria have been developed over the past decades, the most recent being the McDonald 2017 criteria (26). According to the McDonald 2017 criteria, a diagnosis of MS requires that an individual must have widespread evidence of CNS damage both disseminating in time and location, meaning the lesions appear in multiple regions of the nervous system and happen at one or more points in time (26). The McDonald criteria for MS diagnosis was a breakthrough in diagnosing MS since it uses magnetic resonance imaging (MRI) to identify the presence of lesions. Prior to the McDonald criteria, the Poser criteria, published in 1983, were widely used for diagnosing MS. These criteria also required lesions to occur at different time points (by a minimum of one month) with varying afflicted areas of the CNS (27); the Poser criteria required using lumbar punctures to analyse spinal cord fluid for protein bands (27).

2.3 Risk Factors for Multiple Sclerosis

MS is a disease with peak onset usually in the late 20's and early 30's and is most common amongst individuals of European descent (4). Women have a higher prevalence of MS than men worldwide, with female:male prevalence ratios varying between 1.5:1 to 2.5 (4). There are several risk factors for MS which have been explored in depth throughout the past decades. The following will be discussed in this section: genetics, geography, EBV, vitamin D deficiency through sun exposure and diet, cigarette smoking, and obesity.

2.3.1 Genetics

As previously mentioned, one factor which increases the risk of MS is genetic predisposition, with incidence and prevalence grouping at a higher rate in families compared to the general population. Twin and familial studies have shown that monozygotic twins have a higher concordance rate of MS, ranging between 25-30%, compared to dizygotic twins, and that family history of MS is reported in 15-20% of MS cases (28). The lifetime risk of MS is estimated at 3% for first-degree relatives of MS cases, which is a threefold greater risk than the age-adjusted risk for second- and third-degree relatives and a 10- to 30-fold greater risk than the age-adjusted general population (28). Studies have identified the human leukocyte antigen (HLA) gene cluster, which encodes for glycoproteins involved in immune regulation, on chromosome 6p21 as the most likely candidate genetic locus for MS (28). Over several decades, it was observed that carrying HLA-DRB1*1501 was associated with a threefold increased odds of developing MS (29). In countries with the highest risk of MS, the frequency of the HLA-DRB1*1501 allele is estimated to be between 14-30% of the population (2).

2.3.2 Geography of MS and The Latitude Gradient

In 2008, the World Health Organization (WHO) established the Atlas of MS to help fill the gaps in MS prevalence data worldwide. According to their 2020 report, the number of cases of MS worldwide was estimated at 1 in every 3,000 people, with some countries reaching estimates as high as 1 in 300 individuals having MS (San Marino and Germany) (3). In 2013, several systematic reviews were published from the same research group which attempted to systematically evaluate the worldwide incidence and prevalence of MS through reviewing published population-based studies (30, 31). One of the reviews explored the incidence and prevalence of MS in the Americas and found that Canada was the most studied region, with crude prevalence in individual regions of Canada ranging from 56.4/100,000 to 298/100,000 individuals (30). In the United States, the coverage of studies was low, and age-standardized prevalence estimates of MS ranged from 29.9/100,000 to 191.2/100,000 individuals (30). Another of the reviews explored the incidence and prevalence of MS in Europe; in the Italian Peninsula, it was found that prevalence ranged from 15.8/100,000 to 197.8/100,000 cases of MS. The region of Sardinia has a markedly higher prevalence of MS compared to other regions, likely due to unique genetic and environmental factors (31). In 2015, the average prevalence of MS in Sardinia was estimated at 299/100,000 individuals, compared to 176/100,000 people in mainland Italy and Sicily (32). In the same previously mentioned systematic review of MS prevalence in Europe published in 2013, it was found that in the Nordic region, the prevalence of MS in more recent studies from Norway, Denmark, and Sweden reached 150/100,000 individuals or greater (31). Other regions, such as the British Isles and the combined regions of Belgium and France also saw high estimates of MS prevalence ranging from 96/100,000 to 200/100,000 and

80/100,000 to 149/100,000 individuals, respectively (31). In countries located closer to Central and South Eastern Europe, the prevalence of MS was generally lower (31).

One phenomenon described in MS epidemiology is the latitude gradient of MS prevalence and incidence; this refers to the phenomenon whereby MS prevalence and MS incidence increase as one moves further away North or South from the equator (33). A systematic review published in 2008 assessed studies on the incidence of MS published between 1966-2007 and found that for each increment of 10 degrees away from the equator, the incidence of MS increased by 30% in women and 50% in men (although women altogether had higher incidence estimates than men) (34). They found this gradient to be attenuated after the year 1980, when countries in lower latitudes saw an increase in MS incidence (34). A systematic review and meta-analysis published in 2019 examined the association between MS prevalence and latitude in studies published up to 2010; they found latitude to be consistently associated with an increased risk of MS away from the equator, North or South (33). In region-specific analyses, they found a "statistically significant" increasing prevalence gradient in areas of European descent, particularly Australia, UK/Ireland, and North America (33). A decreasing prevalence gradient was found in Italy, which is attributed to a regional variation in HLA-DRB1 allele frequencies (33). The latitude gradient in MS incidence is thought to serve as indirect evidence that a lack of vitamin D through sun exposure is linked to an increased risk of MS (33).

2.3.3 Epstein-Barr Virus

It has long been suspected that an infectious agent may be involved in the causal pathway of MS (35). One compelling draw to the infectious agent hypothesis is that it may help explain the geographic variability in the risk of MS, as well as the change in MS risk linked to migration (35). Several studies have shown compelling epidemiological and serological evidence that

infection with EBV, a ubiquitous human lymphotropic herpesvirus, may play a leading role in the onset of MS (35). A systematic review and meta-analysis, published in 2013, examined the risk of developing MS for individuals who are seronegative for EBV (36); They found that only 1.7% of adult MS patients were seronegative for EBV, compared to 6.3% of controls, and that the overall odds ratio for MS was 0.18 (0.13-0.26) in individuals seronegative for EBV across 22 studies (36). More recently, in a large cohort study published in 2022 of over 10 million young adults in active duty for the US military followed over 20 years, it was found that the risk of MS was increased by 32-fold following EBV infection while the risk of MS was low in individuals who have never been infected with EBV (37). A systematic review and meta-analysis of the association between EBV and MS, published in 2020, found that the odds of MS in individuals with high anti-EBV antibodies is increased in those with the HLA-DRB1*1501 gene, which is the gene previously established as increasing the risk of MS, compared to those without the gene (38). This could point to an interaction between the anti-EBV antibody titres and HLA genotypes on the additive scale (38). According to this meta-analysis, studies on EBV seropositivity and MS show that EBV seropositivity was more common in people with MS compared to controls (OR = 3.92, CI = 3.10-4.96) (38).

A common hypothesis, known as the “hygiene hypothesis”, postulates that individuals with multiple infectious exposures in early childhood have a reduced risk of disease, including MS (39). In fact, even by 1966 it was shown that the risk of MS is higher in individuals who had a high degree of hygiene/sanitation (meaning low infections) in childhood (39). One clinical manifestation of EBV, particularly in adolescence, is infectious mononucleosis (IM) (40). It was initially believed that MS and EBV were not causally linked to one another, but rather shared similar etiology (35). Based on the hygiene hypothesis, we would expect individuals with low

infectious exposures in childhood to have an increased risk of MS. Similarly, we would expect individuals with a high hygiene in childhood to have contracted EBV later in life, such as adolescence, which would be more likely to result in the clinical manifestation of IM. Therefore, it seems that high hygiene in childhood could potentially confound the association between EBV and the increased risk of MS (35). A cohort study from Sweden, published in 2021, demonstrated that not only is EBV associated with a higher risk of MS, but that adolescence is specifically an important period of susceptibility between IM and MS (41). In their population-based cohort sibling study of over 2,000,000 individuals, they found that IM in adolescence (HR: 3.00; 95% CI: 2.48-3.63) was more highly associated with an increased risk of MS compared to IM in childhood (HR: 1.98; 95% CI: 1.21-3.23) (41). Therefore, this demonstrates that EBV and its clinical manifestation IM, and not high hygiene in childhood, is likely linked to the increased risk of MS. The mechanism through which EBV may increase the risk of MS is unknown, although there is evidence that EBV may be a 'trigger' or 'driver' in MS pathogenesis which greatly increases the risk of MS, particularly when afflicted in adolescence (42).

2.3.4 Vitamin D Deficiency: Diet and Sun Exposure

It has been shown in studies for years that low vitamin D exposure, both through sunlight and diet, is associated with an increased risk of MS (43). As previously mentioned, the existence of the latitude gradient in MS incidence potentially serves as evidence of the link between vitamin D, sun exposure, and MS whereby individuals living in regions with warmer climates receive higher sun exposure and have a lower risk of MS (33). Skin exposure to ultraviolet (UV)-B radiation is a major source of vitamin D in humans and helps to make up to 80-90% of the body's vitamin D supply (44). In the Nurses' Health Study in the United States, it was found that individuals living in high UV-B areas before MS onset had a 45% lower risk of MS compared to

individuals living in low UV-B areas (45). It was shown in a study published in 2006 that individuals have a decreased risk of MS when they have increased levels of vitamin D, both from diet and sun exposure, particularly when exposure occurs prior to the age of 20 years (46).

In a systematic review of early life vitamin D exposure and the later risk of MS, published in 2019, it was reported that MS was more common in individuals born in April and May in the Northern Hemisphere, and less frequent in those born in November and December, and the inverse relationship was observed in the Southern Hemisphere (meaning gestation in both these cases would have occurred during low-sun exposure months) (47). These findings suggest that a higher exposure to UV radiation during gestation is linked to a lower risk of MS (47). Studies also found reasonable evidence of a modulated risk of MS in migrants who move from a low to high risk or high to low-risk areas, particularly prior to the age of 15 years (47); This association is thought to partly stem from early life vitamin D exposure, whereby someone moving from an area of high vitamin D to low vitamin D increases their risk of MS. This also indicates the importance of vitamin D exposure in early life on the risk of MS. A study using data from EnvIMS, published in 2019, showed that groups with low sun exposure and high sun protection use prior to the age of 15 are associated with an increased risk of MS (RR: 1.76; 95% CI: 1.27-2.46) compared to groups with high sun exposure and low sun protection use (48). It has been theorized that a higher level of vitamin D helps to reduce the immune system's inflammatory response which reduces the risk of autoimmune disorders, including MS (47). Past research has found that the gene responsible for MS susceptibility, HLA-DRB1*1501, is regulated by a vitamin D-dependent promotor, which shows that vitamin D could continue to have a clinically relevant effect on the risk of MS even independent of sun exposure and UV-B (44).

The other 10-20% of the body's vitamin D supply is obtained through diet and supplementation (44). One Scandinavian study looking at whether serum 25-hydroxyvitamin D (25[OH]D) levels in early pregnancy are associated with increased risk of MS in offspring found that low levels could be linked to a nearly 2-fold increased risk of MS (49). In the Nurses' Health Study, it was found that those who had a higher intake of dietary vitamin D had a 33% lower incidence of MS compared to those with a lower intake, and that those who used vitamin D supplements had 41% less risk of developing MS compared to those who did not use vitamin D supplements (50).

2.3.5 Cigarette Smoking

Cigarette smoking as a risk factor for MS can be defined under two categories: smoking and second hand or passive smoke exposure. The relationship between smoking and the increased risk of MS is believed to be dose- and duration dependent and individuals who smoke also risk experiencing a rapid progression of MS (51). A 2011 systematic review and meta-analysis on the risk of MS in smokers found, across 10 studies, that smoking was associated with a 48% higher MS susceptibility compared to non-smokers (52). One Swedish study published in 2009 attempted to distinguish the effect of tobacco smoking from use of snuff, another type of tobacco product, and saw that only smoking was associated with an increased risk of MS and not snuff (53). Smoking has also been shown to interact with the HLA-DRB1*1501 gene where smokers carrying the MS-associated gene were shown to have an up to 13-fold increased risk of MS compared to non-smokers (54). In terms of second-hand smoke, several studies have also shown that exposure to passive smoke, particularly in adolescence, is associated with an increased risk of MS (55-57).

2.3.6 Obesity

High BMI and obesity have been linked to an increased risk of MS, particularly when it occurs in adolescence (43). A study using EnvIMS data in Italy and Norway, published in 2015, used Stunkard's standard body silhouettes, which range from one (smallest) to nine (largest) body sizes to examine if body size is associated with the risk of MS (58). They found that larger body silhouettes (silhouettes 6-9) from ages 10-30 years were associated with an increased risk of MS, with the strongest effect being shown around the age of 25 years (58). In another Norwegian cohort study, published in 2021, it was found that obesity, particularly in the younger age groups between the ages of 14-24 years, is associated with higher risk of MS later in life in both men and women (59). Interestingly, findings from a cohort study in the United States showed that obesity in young females was associated with an increased risk of pediatric MS onset (60). As with other described risk factors, some interaction between obesity and the HLA-DRB1*1501 gene further increased the risk of MS in obese individuals, particularly when obesity occurred in adolescence (61).

2.4 Autoimmune Disorders and Multiple Sclerosis

The association between MS and other AiDs has been a growing area of interest; any link between AiDs and MS could help uncover common genetic or environmental exposures and help to better understand the etiology of MS (9). A 2015 systematic review on the incidence and prevalence of AiDs in PwMS reported that the prevalence of AiDs across four studies (the exact AiDs varying by study) ranged from 3-26.1% (9). A literature search was conducted in order to identify the existing studies on the association between the nine AiDs of interest in this thesis and the risk of MS. This search is described in section 2.4.1.

One case-control study by Zorzon et al. was identified in the search for assessing the risk of MS associated with several risk factors of MS, including having an AiD (62). This paper cannot be included in the literature summary below since they did not define which AiDs were being reported by participants in their study; however, it is interesting to note that Zorzon et al. reported that having an AiD prior to MS diagnosis (and similar period in controls) was associated with an increased risk of MS (OR = 6.8; 95% CI = 1.4-32.0) (62). While the results of this study cannot be compared to any future analysis which will be performed in Chapter 4 since there was no information on which specific AiDs were reported by participants, it is noteworthy that this study suggests some association between AiDs and the increased risk of MS (62).

2.4.1 Risk of MS in People with Specific AiDs

To more formally identify the existing body of research on the risk of MS in people with specific AiDs, a search was run on Medline. Studies were included if they were observational studies published between January 1st, 2000, and July 7th, 2022. To be included, the occurrence of at least one of the nine previously identified AiDs had to clearly precede the outcome of a diagnosis of MS and the study needed to report the measure of association as either an odds ratio (OR), relative risk (RR), incidence rate ratio (IRR), risk ratio, or hazard ratio (HR) of MS. Studies published prior to year 2000 were excluded to prevent including studies that used outdated diagnostic criteria for MS. The initial search yielded 5,202 publications. The references of relevant studies, and of systematic and narrative reviews on the topic, were also searched for additional publications. After rounds of title, abstract, and full text screening, a final four eligible cohort and five eligible case-control studies were included (10-18). Figure 2.1 and Figure 2.2 show the PRISMA chart summaries of study exclusion from this search. Three of these publications were identified from the references of other studies identified in Medline. These

studies were not originally identified in the search since they do not have any of the nine AiDs listed under their MeSH terms and therefore were not picked up by the search.

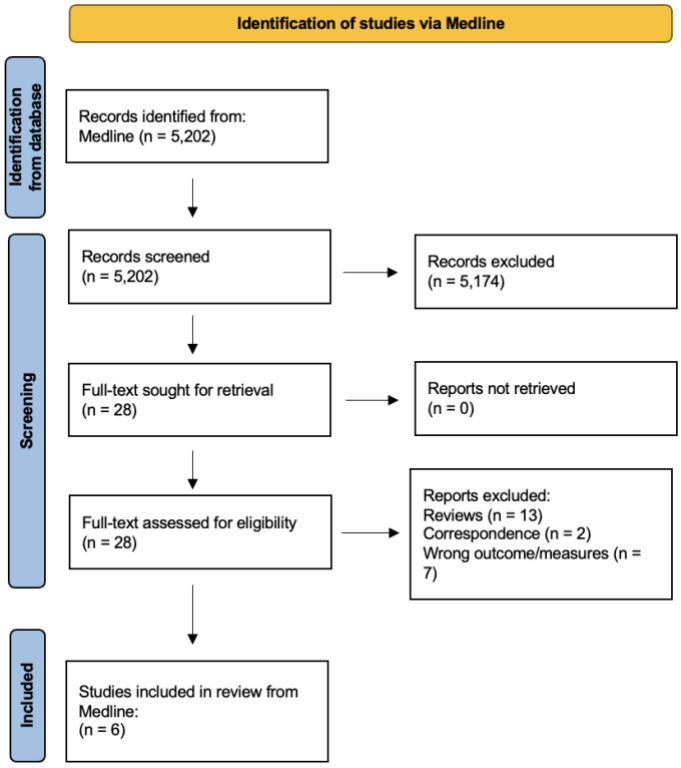

Figure 2.1. PRISMA flow diagram of studies identified from Medline for background literature search.

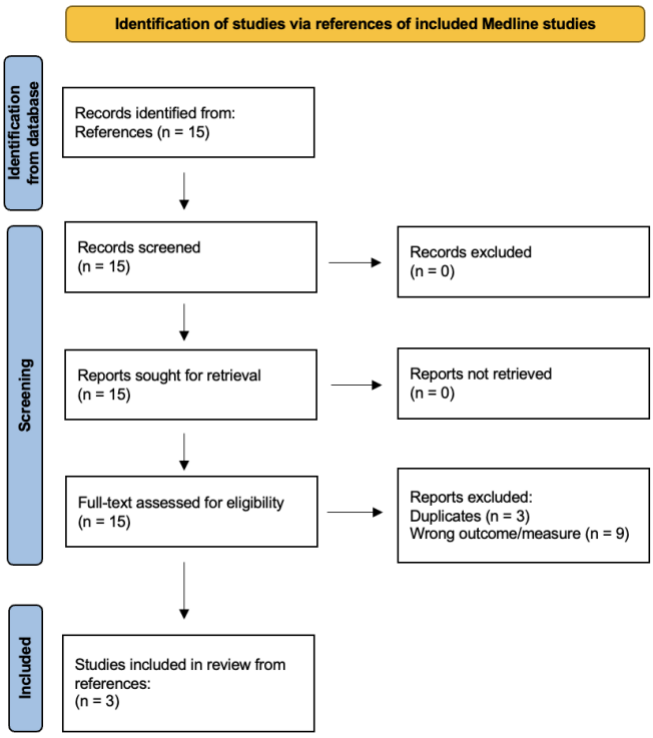

Figure 2.2. PRISMA flow diagram of studies identified from the references of included Medline for background literature search.

As previously mentioned, the AiDs that are the focus of this research are T1D, psoriasis, SLE, RA, CD, UC, celiac disease, hypothyroidism, and hyperthyroidism. The studies were published between 2006-2021, with three being from Iran, three from Denmark, one from Sweden, one from South Korea, and one from the United States. Summaries of the results from these studies are presented in Table 2.1 (Case-Control studies) and Table 2.2 (Cohort studies).

Quality Assessment: Case-Control Studies (N = 5)

In order to provide high-quality evidence on the association between AiDs and MS, a case-control study should have the following: 1) a well-defined primary base of MS cases according to well-established diagnostic criteria for MS, 2) a sample of controls that is representative of the case source population, 3) clear criteria for ascertaining pre-MS diagnosis of AiDs and their timing (either through self-report or medical records) that is identical for cases

and controls, 4) clearly defined exposure periods for both cases and controls. Five case-control studies were included from the search. Of these studies, only Langer-Gould et al. (2010) specifically looked at whether a number of AiDs increased the risk of MS (13). Magyari et al. (2014) also assessed the risk of MS in people with AiDs, although they specifically looked at if this risk differed between males and females (15). Abbasi et al. (2017) assessed the association between various environmental factors, including AiDs, and the risk of MS (10). Abdollahpour et al. (2019) explored how medical history, including AiDs, affects the risk of MS (11). Finally, Maroufi et al. (2021) also looked at how medical history, including AiDs, affects the risk of MS, although they restricted their analysis only to PPMS (16).

Case selection and ascertainment

The ascertainment of cases of MS was considered of good quality if it was determined by a neurologist using the McDonald or Poser criteria (26, 27), if it was obtained through hospitals or specialized clinics for MS, or if it was obtained through a hospital-based or national MS registry (63). Magyari et al. recruited incident cases of MS from the Danish MS Registry and included all individuals who had been diagnosed with MS according to the McDonald criteria (15). Langer-Gould et al. used the Kaiser Permanente Northern California (KPNC) medical care program database to identify incident and prevalent cases of MS in California using MS diagnostic codes by neurologists and primary care physicians (13). Abbasi et al. recruited prevalent cases of MS which had been referred to university hospitals in Iran and were diagnosed according to the McDonald criteria for MS, although they do not specify if diagnosis was performed by a neurologist (10). Abdollahpour et al. recruited incident cases with a confirmed diagnosis of MS by at least one neurologist according to the McDonald criteria using the Iranian Multiple Sclerosis Society Registry in Tehran, Iran (11). Finally, Maroufi et al. recruited prevalent cases

of PPMS diagnosed by a neurologist according to the McDonald criteria and referred to Sina Hospital in Tehran, Iran (16). Individuals with a cognitive disorder or memory dysfunction were excluded (16).

Within case selection, all studies adequately described the source of their cases and described how MS was ascertained. Abbasi et al. was the only study who did not specify that MS was diagnosed by a neurologist. Langer-Gould et al. stated that diagnostic codes of MS could have either been inputted by neurologist or a primary-care physician (ideally, MS should be diagnosed by a neurologist). While four of the studies looked at the effect of AiDs on the risk of MS, Maroufi et al. limited their study to only individuals with PPMS. Since PPMS only makes up ~10-15% of all diagnoses of MS, it is difficult to compare the results of this study to the others (20, 21).

Control selection

Control selection is an important factor to consider when reviewing the methods of a study as poor control selection can introduce selection bias and possibly distort the results of a study. General guidelines for control selection suggest that controls should be representative of the same base experience as cases, that exposure to unmeasured factors have as little variability as possible, and that the degree of accuracy in measuring exposures be the same for cases and controls (64). It is also important that authors describe whether and how MS was ruled out in controls.

Magyari et al. used the Danish Civil Registration System to identify population-based controls that were matched to cases on sex, year of birth, and residential municipality, and cross referenced with the Danish MS Registry to ensure that controls had not been diagnosed with MS (15). Abdollahpour et al. recruited population-based controls through random digit dialing

(RDD) in the same region where cases were recruited (11). Controls were described as "non-diseased", although no further information is provided on how this was established. Langer-Gould et al. recruited controls from the same medical database through which they recruited their cases and matched based on gender (sic), birth year (± two years), KPNC facility, and duration and timing of KPNC membership (13). They do not mention if or how they ruled out MS in controls. Abbasi et al., which recruited cases from hospitals, enrolled as controls the healthy relatives of patients referred to other hospital departments during the same time period as the cases (10). Controls were matched to cases on age and sex. They do not describe which hospital departments they recruited from, nor if the controls were spousal relatives or biologically related to the hospital patients (10). They also do not describe how they established that controls were "healthy". Finally, Maroufi et al. recruited population-based controls via RDD performed within the same region as cases (16). Controls were matched to cases on sex and were required to have no history of neurological disorders and anyone with cognitive disorders or memory dysfunction were excluded (16).

Magyari et al. stands out as having appropriately recruited controls from the same primary base as their cases and ruling out MS in controls. Abdollahpour et al. and Langer-Gould et al. also recruited controls from the same primary base as cases, although both fail to mention how MS was ruled out in the controls. Abbasi et al. do not provide enough information on which hospital departments they recruited the healthy relative controls from, and they do not explain whether the controls were spousal or biological relatives. Finally, Maroufi et al. state that their controls had no history of any neurological disorders. It is unclear whether this constitutes an exclusion criterion or if it is simply a feature of the control cohort. They do not state that the same criterion of having no neurological disorders other than MS was applied to cases, meaning

the controls may not be representative of the source population of cases. They do state that both cases and controls with cognitive disorders or memory dysfunction were excluded, although it is unclear if this means that other neurological disorders were ruled out in cases in the same way that they were in controls. This could be an issue if an exclusion criterion that was imposed on the controls was not applied to the cases.

Ascertainment of exposure and exposure periods

For each of these studies, it is important that temporality between the diagnosis of the AiDs and the diagnosis of MS be clearly established. Additionally, we want there to be a clearly defined comparable exposure period for cases and controls.

Two studies benefitted from using databases to access information on AiD diagnosis. Magyari et al. used the Danish National Patient Register to identify AiDs in both cases and controls through International Classification of Diseases (ICD) codes dating back to 1977 (15). The diagnostic sequence between the AiDs and MS was established by reviewing the first year of AiD diagnosis relative to the clinical onset of MS (which subsequently became the reference year for the controls as well) (15). Among our nine AiDs of interest, Magyari et al. included T1D, RA, CD, UC, SLE, Graves' disease (a common cause of hyperthyroidism), and autoimmune thyroiditis (a common cause of hypothyroidism) (15). Langer-Gould et al. utilized the KPNC medical database to ascertain exposure of AiDs in cases and controls and used electronic clinical records to identify individuals with at least two codes entered by a medical specialist (13). They analyzed incident cases of MS separately from the prevalent cases in order to assess the timing of diagnoses and determined that an AiD preceded MS if the first code for the AiD came before the first code for MS (first code defined as a code for either MS, optic neuritis, transverse myelitis, or central nervous system demyelinating disorder) (13). It is unclear

what the exposure period was for controls. The AiDs included in this study that are of interest are psoriasis, T1D, RA, inflammatory bowel disease (IBD), Hashimoto's thyroiditis, Graves' disease, and SLE (13).

Abbasi et al. conducted face-to-face interviews using structured checklists and a standardized protocol for data collection for both cases and controls (10). Cases were asked to recall their exposures prior to MS onset, and controls were asked about their exposures "within a similar duration of time" (10); it is not stated how this similar duration of time was defined. In their study, Abbasi et al. included T1D, thyroiditis, and IBD (10). They were not able to provide an estimate for IBD, although they do not elaborate on the reason why. Abdollahpour et al. conducted telephone interviews with interviewers trained to use standardized data collection procedures (11). Interviewers were not blinded to participant status; however, interviews were monitored for any interviewer bias by randomly recording calls (11). Participants were queried on their lifetime occurrence of all nine AiDs of interest; the AiD exposure period was considered to be the index date in cases and the sampling date in controls (11). Finally, Maroufi et al. collected information on exposures from cases and controls using the Persian version of the EnvIMS Questionnaire (EnvIMS-Q). They conducted face-to-face interviews for cases and telephone interviews for controls (16); having differing interview methodologies for cases and controls can introduce interviewer bias whereby interviewers may further prompt cases for additional information and better recall of their exposures. Moreover, this also highlights that interviewers were not blinded to participant status, which may additionally lead to interviewer bias. Cases and controls were asked to report the age of diagnosis for each AiD to ensure that they preceded the onset of PPMS (16). They do not describe how the exposure period was defined in controls. All nine AiDs of interest were considered in this study.

When looking at the overall methodologies of the included case-control studies, Magyari et al. stands out for meeting all criteria of high-quality evidence. They enrolled cases from a national MS database which uses the McDonald diagnostic criteria for MS, recruited population-based controls from the same source population as the cases, clearly identified the period of exposure in both cases and controls, and firmly established temporality between the diagnoses of the AiDs and MS. Abdollahpour et al. additionally met all criteria for appropriate case and control selection, although interviewers were not blinded to participant status. Langer-Gould et al. met most of the criteria for high-quality evidence but did not explicitly define the period of exposure considered in controls. Additionally, Abbasi et al. do not adequately describe the exposure period in controls. In their discussion, Abbasi et al. briefly describe considering a time lag between history of exposure and MS diagnosis when no MS-related symptom had begun, although they do not describe the nature of this time lag in their methods and there is no indication of when it was used in their analysis (10). They recruited their controls from the healthy relatives of patients in other hospital departments from where the cases were recruited, although did not provide information on which departments and whether the relatives were spousal or biologically related; It is possible that the patient relatives of the controls were in hospital departments related to one of the AiDs of exposure, meaning these controls could potentially have a genetic predisposition to one of the AiDs which would increase their risk of being afflicted and open up the control group to confounding. Maroufi et al. also did not describe the exposure period in controls and used different interview methods for cases and controls, which may introduce bias in their data collection process (16). They additionally limited their

study to only the PPMS disease course, which limits the comparability of their results to other studies.

Table 2.1. Summary of case-control studies examining AiDs and the risk of MS

Authors (year)	**Country**	**Cases of MS**		**Source of Controls**		**AiDs included**	**Measure of association (95% CI) for AiD**	**Adjustment variables**
		n	*Source*	*n*	*Source*			
Abbasi et al. (2017) (10)	Iran	660	Hospitals	421	Relatives of hospital patients	T1D	OR: 0.11 (0.01-0.99)	Age, sex, education, ethnicity, income, marital status
						IBD	nc	
						Thyroiditis	OR: 0.95 (0.44–2.05)	
Abdollahpour et al. (2019) (11)	Iran	547	Iranian MS Society Registry	1,057	22 areas of Tehran	T1D	OR: 0.38 (0.08-1.80)	Age, sex
						RA	OR: 0.70 (0.37-1.31)	
						Psoriasis	OR: 2.10 (0.60-7.32)	
						SLE	nc	
						CD	OR: 6.56 (0.59-72.73)	
						UC	OR: 6.56 (0.59-72.73)	
						Celiac	nc	
						Hypothyroidism	OR: 1.12 (0.78-1.60)	
						Hyperthyroidism	OR: 0.74 (0.38-1.42)	
Langer-Gould et al. (2010) (13)	United States of America	924	Kaiser Permanente Northern California database	4,620	Kaiser Permanente Northern California database	T1D	OR: 0.8 (0.3-2.4)	Age, gender, KPNC membership duration
						IBD	OR: 2.7 (1.1-6.8)	
						RA	OR: 0.5 (0.1-2.0)	
						Psoriasis	OR: 1.8 (0.9-3.6)	
						SLE	OR: 1.3 (0.4-4.0)	
						Hashimoto's thyroiditis	OR: 5.0 (0.3–80.2)	
						Graves' disease	OR: 0.7 (0.2–2.9)	
Magyari et al. (2014) (15)	Denmark	1,403	Danish MS Registry	35,045	Danish Civil Registration System	T1D	OR: 3.34 (1.40-7.02)[a]	Stratified by sex
						RA	nr	
						Psoriasis	nr	

						SLE	OR: 12.55 (1.62-69.95)[a]	
						CD	OR: 5.03 (1.18-16.10)[a]	
						UC	OR: 2.22 (0.93-4.59)[a]	
						Hashimoto's thyroiditis	nr	
						Graves' disease	nr	
Maroufi et al. (2021) (16)	Iran	143	Hospital	143	22 areas of Tehran	T1D	OR: 0.08 (0-1.81) [b]	Age, sex, marital status, self-rated health
						RA	OR: 0.22 (0.03-1.78) [b]	
						Psoriasis	OR: 7.38 (0.01-6.96) [b,†]	
						SLE	nc	
						CD	nc	
						UC	OR: 0.75 (0.05-10.79) [b]	
						Celiac	nc	
						Hypothyroidism	OR: 3.20 (1.23-8.30) [b]	
						Hyperthyroidism	OR: 0.39 (0.06-2.58) [b]	

OR = odds ratio
nc = not calculated
nr = not reported, calculated but results shown only in a figure
RDD = random digit-dialing
[a] estimate is only for male participants
[b] OR is only for PPMS disease course
[†] OR is not within the 95% CI, this seems to be a typo in the paper

Quality Assessment: Cohort Studies (N = 4)

In order to provide the strongest evidence, cohort studies should include: 1) clear criteria for identifying individuals with the defined exposure, with the exposure being one of our nine AiDs of interest, 2) clear criteria for identifying a comparison group, 3) clear assessment of MS outcome in the cohorts according to well-established criteria for MS diagnosis, 4) clearly defined follow-up time. Four cohort studies were identified from our literature search. Egeberg et al. conducted a study on the risk of MS in patients with psoriasis (12). Ludvigsson et al. explored the risk of neurological disease, including MS, in individuals with celiac disease (14). Nielsen et al. assessed the risk of MS in individuals with T1D (17). Finally, Park et al. looked at the risk of immune-mediated illnesses, including MS, in a cohort of individuals with IBD (which was further broken down to look at the risk of MS in individuals with CD and UC) (18).

Exposed cohorts

Egeberg et al.'s study comprised the entire Danish population 18 years and older; at baseline, they excluded individuals with prevalent psoriasis and MS (12). Therefore, at baseline all individuals were unexposed to psoriasis. Individuals with incident psoriasis (mild or severe) were identified when they filled a second prescription for the first-line treatment of psoriasis in Denmark or by their first in- or out-patient consultation for psoriasis or psoriatic arthritis (12). Information on the diagnosis of psoriasis was accessed through the Danish National Patient Register using ICD codes, while data on prescriptions were accessed using the Danish Registry of Medicinal Products Statistics (12). Ludvigsson et al. used the Swedish National Inpatient Register to identify individuals with a hospital-based discharge diagnosis of celiac disease according to ICD codes (14). They excluded any individuals who had received a diagnosis of MS prior to study entry or within the first year of study entry. Nielsen et al. obtained a cohort of

individuals with T1D using the Danish Hospital Discharge Register; individuals with T1D were identified using ICD codes. They do not describe how or if MS was ruled out at baseline for individuals with T1D enrolled in the study (17). Finally, Park et al. identified a cohort of individuals with CD or UC using insurance claims data from the Health Insurance and Review Agency based on a special co-payment programme code from the rare intractable diseases registration database in South Korea (18).

All four cohort studies appropriately described the source of their exposed cohort and stated how the AiD exposure was ascertained in their cohort.

Comparison cohorts

The comparison cohorts of these studies should be drawn from the same population as the exposed cohorts. It is important that the AiD exposure and MS outcome both be ruled out in the comparison cohorts.

As previously mentioned, in Egeberg et al.'s cohort, psoriasis and MS were both ruled out at baseline. Therefore, anyone who did not develop psoriasis during the follow-up period comprised the comparison cohort (12). Ludvigsson et al. identified their comparison cohort using the Total Population Register in Sweden, whereby up to five reference individuals were identified for each person with celiac disease, matched on age, sex, calendar year, and area of residence (14). They excluded any reference individual who had a prior diagnosis of MS at study entry (14). For their comparison cohort, Nielsen et al. calculated the expected number of MS cases in their cohort as the sum of sex-, age-, and period-specific person-years at risk in the T1D cohort multiplied by the national sex-, age-, and period-specific MS incidence rates available from the Danish MS Register (17). Finally, Park et al. describes enrolling up to four non-IBD controls for every individual with IBD in their cohort, matched on age and sex, using the Health

Insurance and Review Agency database in South Korea (18). In both the exposed and comparison cohorts, they excluded anyone who had a diagnosis of one of the co-immune-mediated illnesses, including MS, during the study inclusion period (18). All four cohort studies had an appropriately defined comparison group.

Follow-up and ascertainment of MS

Egeberg et al. conducted follow-up from 1997 to 2011, with participant follow-up ending either at MS diagnosis, emigration, or death (12). Data on MS diagnosis were obtained from the Danish National Patient Register and based on ICD codes (12). They were able to establish timing between diagnoses of psoriasis and MS since both illnesses were ruled out in participants at baseline (12). Ludvigsson et al. followed up participants starting from 1964 until either the date of first discharge diagnosis for a neurological disorder, emigration, death, or the end of the study period in 2003 (14). Participants could be enrolled in the study if they were diagnosed with celiac disease at any time between 1964-2003, although were removed if they were followed up for less than a year (14). Diagnosis of MS was ascertained through ICD codes in the Swedish National Inpatient Register (14). Nielsen et al. identified their T1D cohort dating back to people diagnosed in 1977 and continued to follow-up until either MS diagnosis, death, emigration, or the end of follow-up in 1997. Diagnosis of MS was ascertained through the Danish MS Register by linking it to the Danish Hospital Discharge Register (17). Finally, Park et al. followed individuals with IBD between 2012-2016 and used ICD codes in the Health Insurance and Review Agency database to identify incident cases of MS (and other immune-mediated illnesses) (18).

During follow-up, Egeberg et al. and Nielsen et al. only looked at MS as their outcome of interest. Ludvigsson et al. assessed the risk of multiple neurological conditions, including MS,

while Park et al. looked at the incidence of several immune-mediated conditions, including MS, in their cohort. In their analysis of the risk of MS, Ludvigsson et al. only excluded individuals who had received a diagnosis of MS prior to study entry and did not exclude based on their other outcomes of interest. Meanwhile, Park et al. excluded anyone during inclusion who had a previous diagnosis of one or more of their nine immune-mediated illnesses of interest (including MS).

The overall methodologies of the cohort studies show that the studies met the criteria for good quality evidence. Egeberg et al. stands out as having used the entire population of Denmark through national registries for their studies, clearly ruling out MS and psoriasis at baseline, and using well-established criteria for MS diagnosis. Interestingly, Egeberg et al. is the only study to adjust for another AiD (T1D) in their analysis. Ludvigsson et al. also used a national inpatient database to identify their exposed cohort and a national population register to obtain a population-based comparison cohort. They ensured that no individuals had MS at baseline and used the inpatient register to identify MS. As their comparison, Nielsen et al. calculated the expected number of causes of MS in their T1D cohort. The Danish MS Registry contains almost all data on Danish citizens with MS onset since 1948 and is annually linked with the Danish Civil Registration System, and therefore is a good choice for calculating the expected cases of MS in a cohort (65). Park et al. identified their cohort of individuals with IBD (including CD and UC) from a national health insurance database in South Korea which reports usage for all citizens in the country. They established temporality between IBD and MS by reporting on incident cases of MS in the cohort and excluded participants with co-immune mediated diseases during the inclusion period.

Table 2.2. Summary of cohort studies examining AiDs and the risk of MS

Authors (year)	**Country**	**AiD population**		**Reference population**		**AiDs included**	**Measure of association (95% CI)**	**Adjustment variables**
		n	*Source*	*n*	*Source*			
Egeberg et al. (2016) (12)	Denmark	58,628 (mild psoriasis) and 9,952 (severe psoriasis)	Danish National Patient Register	5,397,122	Danish National Patient Register	Mild psoriasis Severe psoriasis	IRR: 1.84 (1.46-2.30) IRR: 2.61 (1.44-4.74)	Age, sex, socioeconomic status, smoking, IBD, treatment with statins, therapy with UV light, treatment with TNF-a inhibitors, T1D
Ludvigsson et al. (2007) (14)	Sweden	14,371	Swedish National Inpatient Register	70,096	Total Population Register (Sweden)	Celiac	HR: 0.9 (0.3-2.3)	Regression was conditioned on risk-set defined by sex, age, year of study entry and county of residence
Nielsen et al. (2006) (17)	Denmark	6,078	Danish Hospital Discharge Register	*	*	T1D	RR: 3.26 (1.80-5.88)	Used age-, sex-, and period-specific incidence rates
Park et al. (2019) (18)	South Korea	11,803 (CD) and	Health Insurance	142,324	Health Insurance and	CD	HR: 10.73 (1.11-103.8)	Unclear†

	23,737 (UC)	and Review Agency Database	Review Agency Database	UC	HR: 1.60 (0.39-6.61)

IRR = incidence rate ratio
RR = relative risk
HR = hazard ratio
* = there is no unexposed population, but rather the sex-, age-, and period-specific MS incidence rates from the Danish MS Registry were used to calculate the expected number of MS cases in the cohort
† = it is unclear in this study if they adjusted in their analysis. They mention matching on age and sex and using a multivariable Cox regression model, but do not mention adjusting.

Overall findings from case-control and cohort studies

In terms of case-control studies, Magyari et al. met all of our criteria for providing high-quality evidence (15). Abdollahpour et al. and Langer-Gould et al. met most of our criteria for providing high-quality evidence on the association between AiDs and the risk of MS, although Abdollahpour et al. did not blind interviewers to participant status and Langer-Gould et al. did not adequately clarify their exposure period in controls (11, 13). Meanwhile, Abbasi et al. did not adequately describe the period of exposure in their controls and also did not provide enough information on how controls were recruited (10). Maroufi et al. only considered individuals with diagnosis of PPMS, which reduces the comparability of their results (16). Meanwhile, within the four identified cohort studies, all met the criteria for providing good evidence (12, 14, 17, 18). In this summary, reporting of results will only be done on conditions for which a meaningful analysis could be conducted in the respective study. In some cases, a study could not provide an estimate for a specific AiD due to insufficient sample size. We summarize the results of the Maroufi et al. study separately at the end since they only considered individuals diagnosed with PPMS.

Overall, five studies looked at the association between T1D and the risk of MS (10, 11, 13, 15, 17). In the case-control studies, Magyari et al. found an increased risk of MS in males with T1D (15), while Abbasi et al. found a decreased risk of MS in those with T1D (10), and the rest found no association (11, 13). In their cohort study, Nielsen et al. found that there was an increased risk for MS in a cohort of individuals with T1D compared to the expected number of cases based on incidence calculations (17). Three of the case-control studies looked at psoriasis and SLE and the risk of MS (11, 13, 15). Magyari et al. was the only of the three to find an increased risk of MS in males in association with SLE (15). No association between SLE or

psoriasis and the risk of MS was found across the other studies (11, 13, 15). In their cohort study, Egeberg et al. found an increased risk of MS in individuals with psoriasis compared to the general population (12). Two case-control studies explored the association between CD and UC and the risk of MS (11, 15). While Magyari et al. found an association between CD and the increased risk of MS in males, no other association between CD and the risk of MS was found in the other study (11, 15); no association between UC and the risk of MS was found in either study. The cohort study reported by Park et al. found that people with CD, but not UC, had an increased risk of MS compared to reference individuals (18). Two case-control studies assessed the association between IBD and the risk of MS (10, 13); while Langer-Gould et al. observed an increased risk of MS associated with IBD, it is difficult to determine whether this association is due to the effect of CD or UC since IBD cohorts are usually comprised of an equal proportion of individuals with either illness (66). Four studies looked at either hypothyroidism, Hashimoto's thyroiditis, or thyroiditis, and the risk of MS, all of which found no association (10, 11, 13, 15). Of the three studies which looked at either hyperthyroidism or Graves' disease and the risk of MS, none found an association (11, 13, 15). Finally, two studies looked at the association between celiac disease and the risk of MS (11, 14). The only case-control study which included celiac disease in their study was unable to provide an estimate due to insufficient sample size (11). In their cohort study, Ludvigsson et al. did not find any increased risk of MS in their cohort of individuals with celiac disease compared to reference individuals without celiac disease in the Swedish population (14).

Finally, we consider the results of the case-control study by Maroufi et al. separately since they only considered individuals diagnosed with PPMS. As previously mentioned, PPMS only makes up ~10-15% of all diagnoses of MS (20, 21). Maroufi et al. found no association

between T1D, RA, psoriasis, UC, and hyperthyroidism and the risk of PPMS (16). They were unable to calculate estimates for SLE and celiac disease due to insufficient sample sizes. Finally, Maroufi et al. found an association between hypothyroidism and the increased risk of PPMS (16)

Conclusion of Past Research

In the analysis in Chapter 4, we assess the association between having an AiD and the subsequent risk of MS. Nine studies were identified that assessed the association between AiDs and the risk of MS using at least one of the nine AiDs that were selected for this thesis research. Within the case-control studies, Magyari et al. stands out for meeting all criteria for high-quality evidence. They found evidence that T1D, SLE, and CD are associated with an increased risk of MS in men. Within the other four case-control studies, limitations included not blinding interviewers to participant status (11, 16), using differing interview methods for cases and controls (16), not adequately defining the exposure period in controls (10, 13, 16), and not providing enough details on control recruitment (10). Additionally, Maroufi et al. only included individuals diagnosed with PPMS, which limits the comparability of their results to the other studies (16). Across these four case-control studies, there was some evidence that IBD and hypothyroidism are associated with an increased risk of MS or PPMS (although this is not consistently observed across all studies) (13, 16). Meanwhile, the cohort studies met all criteria for good quality evidence and demonstrated that psoriasis, T1D, and CD may be associated with an increased risk of MS (12, 17, 18).

Overall, the findings across these nine studies are conflicting, with no clear consistent association being seen throughout. These inconsistent results highlight the need for additional research to clarify the relationship between these nine AiDs and the risk of MS, which could help

point to common genetic or environmental risk factors between the AiDs and MS and help us better understand the etiology of MS.

Chapter 3: The Environmental Risk Factors in Multiple Sclerosis Study

The analysis completed in Chapter 4 uses data from the EnvIMS study. EnvIMS is a multinational case-control study conducted in Canada (2012-2013), Norway (2009-2011), Italy (2009-2010), Sweden (2009-2014) and Serbia (2009-2010) (67). For the purpose of the analysis in Chapter 4, only the data from Canada, Italy, and Norway were used. The sample sizes from Serbia and Sweden (281 and 916, respectively) were judged too small for meaningful independent analysis in the study.

The goal of EnvIMS was to explore the etiology of MS and investigate the role of selected environmental risk factors in MS across geographical locations. A total 2,800 cases and 5,012 population-based controls were enrolled in EnvIMS in the five countries (67). Cases of MS were included if they were over the age of 18 years at the time of the study, had disease onset 10 years or less at the time of sampling, and had a diagnosis of MS according to the McDonald or Poser criteria (26, 27). Cases in Italy were recruited from MS registries in Sardinia, Ferrara, and the Republic of San Marino and in Norway from the Norwegian MS Registry and Biobank (67). In Canada, since no MS registries exist, cases were recruited from MS and neurology clinics in Montreal, Toronto, and Winnipeg (67). Controls in each country were frequency matched to cases on sex and age. In Norway and Italy, controls were recruited from Statistics Norway and the Master File Health System of the region of Sardinia, respectively. In Canada, controls were recruited through RDD. Target enrollment was four controls for every case.

The EnvIMS-Q is a mailed self-administered questionnaire which was initially developed in English and subsequently translated to Italian, Norwegian, Serbian, Swedish, and French Canadian using a formal peer-reviewed process (68). The questionnaire contains six sections: Demographics, Sun Exposure, Diet, Medical History, Smoking Habits and Lifestyle Factors, and

Hormonal Factors (women only). It was created through the combined efforts of the collaborators from all the EnvIMS study sites and was guided by existing literature on the etiology of MS and MS risk factors (67). A copy of the English EnvIMS-Q can be found in the Appendix of this thesis. The feasibility, acceptability, and reliability of EnvIMS-Q were assessed in all five EnvIMS countries and EnvIMS-Q was shown to be cross-culturally acceptable, feasible, and reliable (68). Some questions on demographics and exposures were worded differently based on the country of the questionnaire for higher cultural acceptance. For instance, in Canada, Italy, and Serbia, participants were asked to recall exposure in 5-year age intervals of time, while in Norway and Sweden, the intervals were created to correspond to their education system (67).

The EnvIMS-Q was identical for cases and controls and mailed to the homes of eligible participants; envelopes included the questionnaire, an information letter outlining the goals of the study, brochures and messages of encouragement to participate in the study, and a preaddressed postage-paid return envelope to encourage participation (67). Returned questionnaires were reviewed for errors, inconsistencies, and completeness, and subsequently scanned into electronic format using optical scanning (67). The response percentage among cases and controls was 83% and 59% in Canada, 43% and 21% in Italy, and 70% and 36% in Norway, respectively (67). Ethical approval was obtained for each of the EnvIMS study sites, and approval for the analysis in Chapter 4 was added as well (Canada: McGill University: IRB study n. A08-M78-11B).

Several analyses have been published using the data from EnvIMS. One study using data from Canada, Italy, and Norway that explored the link between early life sun exposure and the risk of MS demonstrated that more time spent indoors during childhood and adolescence, as well as increased use of sun protection, were linked with an increased risk of MS (48). Another study

using the EnvIMS data from Italy, Norway, and Sweden explored the association between physical activity and MS and found that vigorous physical activity was inversely associated with the risk of MS (69). Numerous other studies have been conducted with EnvIMS, looking at the association between various risk factors and MS, including smoking, socio-economic status, vitamin D, body size, and infectious mononucleosis (58, 70-73).

For the analysis in Chapter 4, the EnvIMS data from Canada, Italy, and Norway were used to assess the association between diagnosis of select AiDs and the risk of MS. EnvIMS is a good choice for exploring this relationship since information was collected not only on history of AiDs in cases and controls, but the EnvIMS-Q also provides information on an array of purported MS risk factors across different geographical locations in a standardized manner for both cases and controls.

Chapter 4: Exploring the link between autoimmune disorders and the risk of developing multiple sclerosis: An EnvIMS study [Manuscript]

This manuscript contains an analysis of the association between having an AiD of interest and the risk of MS. This analysis draws its data from the Canadian, Italian, and Norwegian sections of the EnvIMS case-control study. A shortened version of this manuscript will be submitted to the Multiple Sclerosis Journal in order to accommodate the journal word limit.

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Abstract

Background: Past research exploring the effect of autoimmune disorders on the risk of multiple sclerosis (MS) has yielded inconclusive results.

Objective: Examine the association between nine autoimmune disorders (psoriasis, rheumatoid arthritis, type-1 diabetes, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, celiac disease, hypothyroidism, and hyperthyroidism) and the risk of MS.

Methods: Altogether 2,242 cases and 3,992 controls from the Environmental Risk Factors in Multiple Sclerosis study (EnvIMS) were included in a logistic regression analysis to estimate odds ratios and 95% confidence intervals for having any or a specific autoimmune disorder and the risk of MS. This was done considering three defined exposure windows.

Results: Having any of our autoimmune disorders diagnosed any time preceding MS was found to be associated with an increased risk of MS in Canada and Italy (1.47 (1.07-2.03) and 1.36 (1.02-1.82), respectively). This association remained in Canada and Italy when the autoimmune disorders were diagnosed at least five years prior to MS (1.61 (1.13-2.29) and 1.41 (1.03-1.93), respectively). Hypothyroidism was found to be associated with an increased risk of MS in Canada both when diagnosed at any time before MS (1.92 (1.14-3.23)) and at least five years prior to MS (2.24 (1.25-4.01)) and in Italy at any time preceding MS (1.93 (1.12-3.32)). Psoriasis was associated with an increased risk of MS in Canada at any time preceding MS (1.86 (1.03-3.37)).

Conclusion: Having any of our nine autoimmune disorders is associated with an increased risk of MS in Canada and Italy. Additionally, hypothyroidism, and possibly psoriasis, are associated with an increased risk of MS.

4.1 Introduction

Multiple sclerosis (MS) is a chronic autoimmune inflammatory and degenerative disease of the central nervous system (CNS) leading to demyelination and progressive neurological deterioration (1). According to the MS Atlas (2020), 2.8 million people are living with MS worldwide, and more than 330 persons per 100 000 inhabitants are living with MS in countries with the highest prevalence (2). MS is believed to be caused by an interaction between genetic, infectious, and environmental factors. Some lifestyle factors which have been associated with an increased risk of MS include cigarette smoking, obesity/high body mass index (BMI), and low levels of vitamin D through sun exposure and/or diet (3, 4). There is also strong evidence that Epstein-Barr virus (EBV) infection plays a causal role in MS (3, 5). Nevertheless, the exact causes and pathogenesis of MS remain unknown. Among the avenues of potential risk factors for MS that remain to be explored, one understudied relationship is how having a preceding autoimmune disorder (AiD) is associated with the risk of MS. Nine AiDs of interest will be explored in this study for potential association with the risk of MS; they are rheumatoid arthritis (RA), psoriasis, systemic lupus erythematosus (SLE), type-1 diabetes (T1D), Crohn's disease (CD), ulcerative colitis (UC), celiac disease, hypothyroidism, and hyperthyroidism. Across nine studies looking at the association between having one of the nine AiDs of interest and the risk of MS (6-14), it was found that psoriasis, T1D, hypothyroidism, CD, and SLE were associated with an increased risk of MS (7-11, 14). However, results across these nine studies were contradictory and while some did report a link between these AiDs and the risk of MS, other studies did not find similar associations (6-9, 12, 13).

The objective of this analysis was to explore, using data from the Environmental Risk Factors in Multiple Sclerosis (EnvIMS) study, whether having any AiD influences the risk of MS

and if one of the nine aforementioned AiDs increases risk of MS. This was explored considering three exposure windows. Based on the evidence from past studies, we hypothesize that T1D, CD, psoriasis, SLE, and hypothyroidism will lead to an increased risk of MS (7, 9-11, 14). Examining the relationship between pre-existing AiDs and MS may suggest shared genetic and environmental factors leading to MS.

4.2 Methods

Study Design

This study draws on data from the EnvIMS case-control study conducted in Canada, Norway, Italy, Sweden, and Serbia between 2009-2014 (15). Only participants from Canada, Italy, and Norway were used for this analysis as the sample sizes from Serbia and Sweden (281 and 916, respectively) were judged too small for meaningful independent analyses. Cases of MS were included if they were over the age of 18 years at the time of the study, had disease onset within 10 years of sampling, and had a diagnosis of MS according to the McDonald or Poser criteria (15-17). Italian cases were recruited from regional MS registries (Sardinia, Ferrara, and the Republic of San Marino) and Norwegian cases from the Norwegian MS Registry and Biobank (15). Since no regional or national MS registries exist in Canada, cases were recruited from MS and neurology clinics in Montreal, Toronto, and Winnipeg and required clinical confirmation of diagnosis (15). EnvIMS target enrollment was four controls for every case, and controls were frequency matched to cases on sex and age. In Europe, controls were recruited through population-based sources and were cross-checked against MS registries to ensure they did not have a diagnosis of MS. In Canada, controls were recruited through random digit dialing using local telephone area codes (Montreal, Toronto, and Winnipeg). In Canada, self-report of MS in the questionnaire was used to exclude controls with a diagnosis of MS. We additionally

performed checking of MS self-report in controls in Norway and Italy. A total of 17 Canadian controls, 11 Italian controls, and eight Norwegian controls were removed due to self-report of MS diagnosis. Additionally, nine Italian cases were removed from analysis due to missing age of MS diagnosis. The response percentage for cases and controls in EnvIMS was 83% and 59% in Canada, 43% and 21% in Italy, and 70% and 36% in Norway, respectively. For the analyses presented here, the Canadian EnvIMS sample includes 587 cases of MS and 961 controls, the Italian sample includes 698 cases and 1,322 controls, and the Norwegian sample includes 957 cases and 1,709 controls; this is a total of 2,242 cases and 3,992 controls.

The EnvIMS-Q

The EnvIMS Questionnaire (EnvIMS-Q) is a mailed self-administered questionnaire first developed in English and subsequently translated to Italian, Norwegian, Serbian, Swedish, and Canadian French (18). The feasibility, acceptability, and reliability of EnvIMS-Q were assessed in all five EnvIMS countries and EnvIMS-Q was shown to be cross-culturally acceptable, feasible, and reliable (18). The EnvIMS-Q was identical for cases and controls and included six sections: Demographics, Sun Exposure, Diet, Medical History, Smoking Habits and Lifestyle Factors, and Hormonal Factors (women only). Some questions on demographics and exposures were worded differently based on the country of the questionnaire for higher cultural acceptance (18).

Autoimmune Disorders

The EnvIMS-Q collected information on history of nine AiDs: RA, psoriasis, SLE, T1D, CD, UC, celiac disease, hypothyroidism, and hyperthyroidism. Participants were asked to report the date of diagnosis for each AiD as part of the medical history module. In the Canadian

questionnaire participants were also asked to report the age of onset of the AiDs, however, only the diagnosis age was considered for consistency of analysis across countries.

For the analyses, three exposure windows were defined. These are summarized in Figure 4.1. Exposure window one (EW1) was defined as any time preceding the diagnosis of MS. Individuals were considered "exposed" if they received a diagnosis of any of the nine AiD any time *prior* to the age of MS diagnosis or index age in controls. Exposure window two (EW2) was defined as any time at least five years prior to the diagnosis of MS. Since it is often difficult to pinpoint when a disease begins, the 5-year window allows a higher degree of confidence in the temporal ordering of the AiD as an exposure and MS as the outcome of interest. Additionally, if the AiDs truly exert a causal effect on MS, the 5-year latency window allows for a period in which an AiD may contribute to the onset of MS (19). Exposure window three (EW3) was defined as the period prior to the age of 18 years. A review summarizing lifestyle and environmental risk factors for MS reported that the period of childhood and early adolescence is an important exposure period for many risk factors of MS, most notably EBV, obesity, and vitamin D deficiency (3). Therefore, we seek to examine if AiDs diagnosed in childhood or adolescence confer an increased risk of MS. Individuals were thus considered "exposed" if they received a diagnosis of an AiD of interest prior to the age of 18 years.

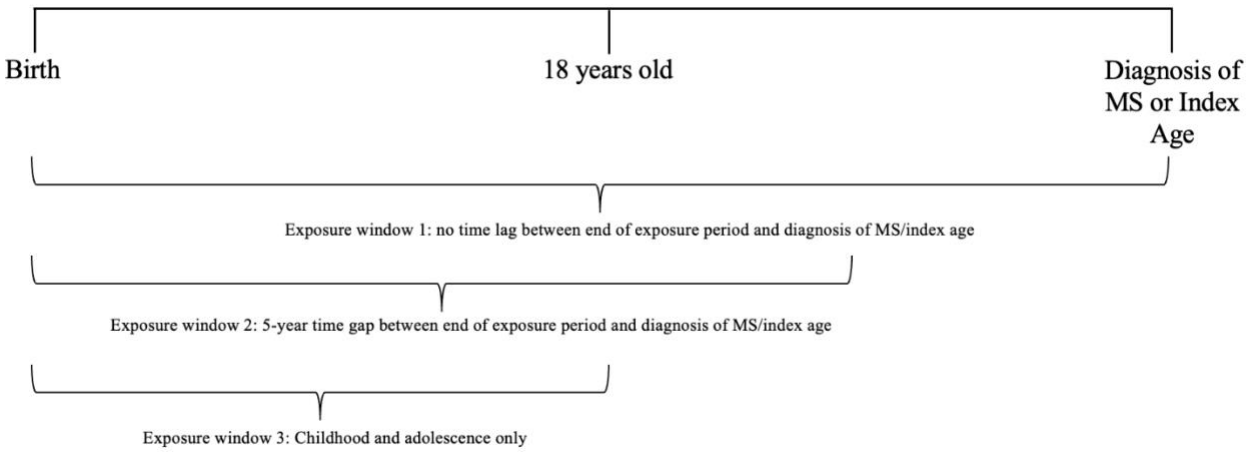

Figure 4.1. Timeline representing the various exposure windows being considered in the analysis of the association between AiDs and MS.

Potential Confounding Variables

Confounders were identified for the association between each AiD and MS. All results for individual AiDs are presented in three ways: crude, age- and sex- adjusted, and "fully adjusted" including additional confounders. Table 4.1 shows the potential confounders identified for each AiD. Our literature search identified the following potential confounders: passive smoke exposure (20, 21), smoking history (22-25), past body size (24, 26-29), history of vitamin D deficiency due to diet or lack of sun exposure (30), and history of EBV (31). In the EnvIMS-Q, exposure to passive smoke was measured through parental smoking inside the home during childhood. Smoking history was defined by how many cigarettes smoked per day between the ages of 11-15, 16-20, 21-25, and 26-30 years (0 cig/day, 1-4 cig/day, 5-10 cig/day, 11-20 cig/day, 25+ cig/day). History of sun exposure was used as a proxy measure for vitamin D deficiency and was measured through report of sun exposure frequency (never, sometimes, often, almost always) in the summer during the same age intervals described for smoking. Past body size, a proxy measure for BMI and obesity, was measured through reported body size at ages 10, 15, 20, 25, and 30 years using Stunkard's body silhouettes ranging from 1-9; four categories of body size were created (1-2 = low, 3 = reference, 4-5 = moderate body size, 6-9 = large body

size). These body silhouettes have been shown to correlate with BMI and are used throughout epidemiological research (32). Similar categorization of these silhouettes, whereby body size 3 was used as the reference size and large body sizes were grouped together as one variable, has been used in previously published EnvIMS research (32). EBV exposure was measured through a self-report of infectious mononucleosis (clinical manifestation of EBV).

Table 4.2.Potential Confounders

Autoimmune Disease	Confounders
Psoriasis	*Passive smoke exposure, History of cigarette smoking, Past body size*
Rheumatoid Arthritis	*Passive smoke exposure, History of cigarette smoking, Past body size*
Systemic Lupus Erythematosus	*History of cigarette smoking, EBV*
Crohn's Disease	*History of cigarette smoking, Past body size*
Celiac Disease	*Vitamin D deficiency*
Hypothyroidism	*Past body size*

Statistical Methods

Logistic regression was used to estimate the crude, age- and sex-adjusted, and fully adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between the AiDs and MS. Two methods of analysis were used. First, the AiDs of interest were combined into a single variable and the association between having *any* AiD and MS was examined. Second, the association between each *individual* AiD and the risk of MS was explored. When looking at each AiD separately, only EW1 and EW2 were used in analysis since too few people were diagnosed with individual AiDs in EW3 for a meaningful analysis to be conducted.

Index ages were calculated for controls based on the MS diagnosis ages of cases in order to identify a comparable period of exposure using an algorithm proposed by Erin Lundy in her MSc Thesis (33); this was done separately for each country. Details of the index age process can be found in the Supplemental and an example of this process using the Canadian data is included

in Supplemental Figure 4.1 (Figure S-4.1). In Canada, controls were older than cases due to a recruitment error during the control selection whereby controls older than 80 were mistakenly recruited. Missing data, including missing ages of AiD diagnosis, were handled by multiple imputation, specifically through the predictive mean matching (PMM) method. PMM imputes the values of missing data based on the observed values in the dataset (34). All variables which were deemed potentially relevant risk factors or confounders were included in the imputation. A total of twenty fully imputed datasets were created and used for analysis. Once the logistic regression models were run, estimates were combined, and 95% CIs were calculated using standard errors computed via Rubin's rule (34).

All statistical analysis was conducted using RStudio Version 2023.03.1+446.

4.3 Results

The demographic breakdown of the participants by country, stratified by case and control, is presented in Table 4.2 (created using the first imputed dataset with no missing values). This includes a count of the AiDs for each country at the time of study and when considering EW1, EW2, and EW3. Cases and controls were predominantly female in all countries. Participants in Italy were younger than those in Canada and Norway. Due to a protocol deviation during control recruitment, Canadian controls were markedly older than the cases (mean age of 48.5 years versus 41.0 years). The highest level of education acquired by participants was similar between cases and controls within their respective countries, with most individuals having achieved "higher education". The most common AiD across all cases and controls was hypothyroidism. In Canada and Italy, more cases reported having *any* AiD than controls. In Norway, this finding was reversed. As exposure window definitions became more restrictive, the number of individuals with each AiD decreased.

Table 4.2. Demographic breakdown of the cases and controls in Canada, Italy, and Norway, and the prevalence of AiDs when considering various time intervals.

	Canada		Italy		Norway	
	Case (N=587)	**Control (N=961)**	**Case (N=698)**	**Control (N=1322)**	**Case (N=957)**	**Control (N=1709)**
Sex						
Male	155 (26.4%)	311 (32.4%)	244 (35.0%)	421 (31.8%)	286 (29.9%)	459 (26.9%)
Female	432 (73.6%)	650 (67.6%)	454 (65.0%)	901 (68.2%)	671 (70.1%)	1250 (73.1%)
Age at time of study						
Mean (SD)	41.0 (10.3)	48.5 (11.4)	38.8 (10.1)	39.3 (10.7)	44.8 (10.5)	46.0 (10.8)
Median [Min, Max]	40.0 [18.0, 68.0]	50.0 [18.0, 68.0]	37.0 [18.0, 66.0]	38.0 [18.0, 79.0]	44.0 [18.0, 80.0]	46.0 [20.0, 86.0]
Highest level of education[a]						
Less than elementary	2 (0.3%)	2 (0.2%)	2 (0.2%)	0 (0%)	21 (2.2%)	45 (2.6%)
Elementary or Middle school	90 (15.3%)	112 (11.7%)	42 (3.2%)	27 (3.9%)	137 (14.3%)	159 (9.3%)
Completed high school	42 (7.2%)	83 (8.6%)	410 (31.0%)	240 (34.4%)	393 (41.1%)	608 (35.6%)
Higher education	452 (77.0%)	761 (79.2%)	868 (65.7%)	431 (61.7%)	406 (42.4%)	897 (52.5%)
Don't know	1 (0.2%)	3 (0.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Frequency of any AiD						
Time of study						
No	471 (80.2%)	803 (83.6%)	543 (77.8%)	1101 (83.3%)	803 (83.9%)	1401 (82.0%)
Yes	116 (19.8%)	158 (16.4%)	155 (22.2%)	221 (16.7%)	154 (16.1%)	308 (18.0%)
EW1						
No	502 (85.5%)	846 (88.0%)	603 (86.4%)	1179 (89.2%)	853 (89.1%)	1514 (88.6%)
Yes	85 (14.5%)	115 (12.0%)	95 (13.6%)	143 (10.8%)	104 (10.9%)	195 (11.4%)
EW2						
No	515 (87.7%)	871 (96.0%)	616 (88.3%)	1204 (91.1%)	866 (90.5%)	1550 (90.7%)
Yes	72 (12.3%)	90 (9.4%)	82 (11.7%)	118 (8.9%)	91 (9.5%)	159 (9.3%)

	Canada		Italy		Norway	
	Case (N=587)	**Control (N=961)**	**Case (N=698)**	**Control (N=1322)**	**Case (N=957)**	**Control (N=1709)**
EW3						
No	565 (96.3%)	933 (97.1%)	667 (95.6%)	1277 (96.6%)	927 (96.9%)	1634 (95.6%)
Yes	22 (3.7%)	28 (2.9%)	31 (4.4%)	45 (3.4%)	30 (3.1%)	75 (4.4%)
Frequency of RA[b]						
Time of study						
No	579 (98.6%)	938 (97.6%)	686 (98.3%)	1274 (96.4%)	940 (98.2%)	1660 (97.1%)
Yes	8 (1.4%)	23 (2.4%)	12 (1.7%)	48 (3.6%)	17 (1.8%)	49 (2.9%)
EW1						
No	583 (99.3%)	952 (99.1%)	688 (98.6%)	1288 (97.4%)	945 (98.7%)	1680 (98.3%)
Yes	4 (0.7%)	9 (0.9%)	10 (1.4%)	34 (2.6%)	12 (1.3%)	29 (1.7%)
EW2						
No	583 (99.3%)	956 (99.5%)	689 (98.7%)	1294 (97.9%)	947 (99.0%)	1685 (98.6%)
Yes	4 (0.7%)	5 (0.5%)	9 (1.3%)	28 (2.1%)	10 (1.0%)	24 (1.4%)
EW3						
No	586 (99.8%)	958 (99.7%)	696 (99.7%)	1311 (99.2%)	954 (99.7%)	1698 (99.4%)
Yes	1 (0.2%)	3 (0.3%)	2 (0.3%)	11 (0.8%)	3 (0.3%)	11 (0.6%)
Frequency of psoriasis[c]						
Time of study						
No	553 (94.2%)	916 (95.3%)	669 (95.8%)	1272 (96.2%)	886 (92.6%)	1591 (93.1%)
Yes	34 (5.8%)	45 (4.7%)	29 (4.2%)	50 (3.8%)	71 (7.4%)	118 (6.9%)
EW1						
No	556 (94.7%)	923 (96.0%)	674 (96.6%)	1285 (97.2%)	904 (94.5%)	1618 (94.7%)
Yes	31 (5.3%)	38 (4.0%)	24 (3.4%)	37 (2.8%)	53 (5.5%)	91 (5.3%)
EW2						
No	565 (96.1%)	931 (96.9%)	677 (97.0%)	1291 (97.7%)	906 (94.7%)	1627 (95.2%)
Yes	23 (3.9%)	30 (3.1%)	21 (3.0%)	31 (2.3%)	51 (5.3%)	82 (4.8%)
EW3						
No	572 (97.4%)	942 (98.0%)	690 (98.9%)	1307 (98.9%)	936 (97.8%)	1668 (97.6%)

	Canada		Italy		Norway	
	Case (N=587)	**Control (N=961)**	**Case (N=698)**	**Control (N=1322)**	**Case (N=957)**	**Control (N=1709)**
Yes	15 (2.6%)	19 (2.0%)	8 (1.1%)	15 (1.1%)	21 (2.2%)	41 (2.4%)
Frequency of SLE[d]						
Time of study						
No	586 (99.8%)	959 (99.8%)	694 (99.4%)	1318 (99.7%)	957 (100%)	1707 (99.9%)
Yes	1 (0.2%)	2 (0.2%)	4 (0.6%)	4 (0.3%)	0 (0%)	2 (0.1%)
EW1						
No	586 (99.8%)	960 (99.9%)	698 (100%)	1319 (99.8%)	1709 (100%)	957 (100%)
Yes	1 (0.2%)	1 (0.1%)	0 (0%)	3 (0.2%)	0 (0%)	0 (0%)
EW2						
No	586 (99.8%)	961 (100%)	698 (100%)	1319 (99.8%)	1709 (100%)	957 (100%)
Yes	1 (0.2%)	0 (0%)	0 (0%)	3 (0.2%)	0 (0%)	0 (0%)
EW3						
No	587 (100%)	961 (100%)	698 (100%)	1320 (99.8%)	1709 (100%)	957 (100%)
Yes	0 (0%)	0 (0%)	0 (0%)	2 (0.2%)	0 (0%)	0 (0%)
Frequency of T1D[e]						
Time of study						
No	583 (99.3%)	956 (99.5%)	684 (98.0%)	1305 (98.7%)	950 (99.3%)	1697 (99.3%)
Yes	4 (0.7%)	5 (0.5%)	14 (2.0%)	17 (1.3%)	7 (0.7%)	12 (0.7%)
EW1						
No	584 (99.5%)	959 (99.8%)	686 (98.3%)	1309 (99.0%)	953 (99.6%)	1700 (99.5%)
Yes	3 (0.5%)	2 (0.2%)	12 (1.7%)	13 (1.0%)	4 (0.4%)	9 (0.5%)
EW2						
No	584 (99.5%)	959 (99.8%)	686 (98.3%)	1311 (99.2%)	953 (99.6%)	1700 (99.5%)
Yes	3 (0.5%)	2 (0.2%)	12 (1.7%)	11 (0.8%)	4 (0.4%)	9 (0.5%)
EW3						
No	585 (99.7%)	960 (99.9%)	691 (99.0%)	1317 (99.6%)	956 (99.9%)	1704 (99.7%)
Yes	2 (0.3%)	1 (0.1%)	7 (1.0%)	5 (0.4%)	1 (0.1%)	5 (0.3%)
Frequency of CD[f]						
Time of study						

		Canada		Italy		Norway	
		Case (N=587)	**Control (N=961)**	**Case (N=698)**	**Control (N=1322)**	**Case (N=957)**	**Control (N=1709)**
	No	582 (99.1%)	955 (99.4%)	695 (99.6%)	1315 (99.5%)	950 (99.3%)	1705 (99.8%)
	Yes	5 (0.9%)	6 (0.6%)	3 (0.4%)	7 (0.5%)	7 (0.7%)	4 (0.2%)
EW1							
	No	584 (99.5%)	958 (99.7%)	696 (99.7%)	1318 (99.7%)	952 (99.5%)	1706 (99.8%)
	Yes	3 (0.5%)	3 (0.3%)	2 (0.3%)	4 (0.3%)	5 (0.5%)	3 (0.2%)
EW2							
	No	584 (99.5%)	958 (99.7%)	697 (99.9%)	1319 (99.8%)	953 (99.6%)	1708 (99.9%)
	Yes	3 (0.5%)	3 (0.3%)	1 (0.1%)	3 (0.2%)	4 (0.4%)	1 (0.1%)
EW3							
	No	587 (100%)	959 (99.8%)	697 (99.9%)	1322 (100%)	956 (99.9%)	1708 (99.9%)
	Yes	0 (0%)	2 (0.2%)	1 (0.1%)	0 (0%)	1 (0.1%)	1 (0.1%)
Frequency of UC[g]							
Time of study							
	No	577 (98.3%)	953 (99.2%)	685 (98.1%)	1302 (98.5%)	951 (99.4%)	1690 (98.9%)
	Yes	10 (1.7%)	8 (0.8%)	13 (1.9%)	20 (1.5%)	6 (0.6%)	19 (1.1%)
EW1							
	No	580 (98.8%)	954 (99.3%)	687 (98.4%)	1310 (99.1%)	952 (99.5%)	1697 (99.3%)
	Yes	7 (1.2%)	7 (0.7%)	11 (1.6%)	12 (0.9%)	5 (0.5%)	12 (0.7%)
EW2							
	No	580 (98.8%)	956 (99.5%)	688 (98.6%)	1311 (99.2%)	952 (99.5%)	1699 (99.4%)
	Yes	7 (1.2%)	5 (0.5%)	10 (1.4%)	11 (0.8%)	5 (0.5%)	10 (0.6%)
EW3							
	No	586 (99.8%)	961 (100%)	695 (99.6%)	1319 (99.8%)	955 (99.8%)	1707 (99.9%)
	Yes	1 (0.2%)	0 (0%)	3 (0.4%)	3 (0.2%)	2 (0.2%)	2 (0.1%)
Frequency of celiac disease[h]							
Time of study							
	No	584 (99.5%)	954 (99.3%)	694 (99.4%)	1314 (99.4%)	955 (99.8%)	1691 (98.9%)
	Yes	3 (0.5%)	7 (0.7%)	4 (0.6%)	8 (0.6%)	2 (0.2%)	18 (1.1%)

		Canada		Italy		Norway	
		Case (N=587)	**Control (N=961)**	**Case (N=698)**	**Control (N=1322)**	**Case (N=957)**	**Control (N=1709)**
EW1							
	No	587 (100%)	958 (99.7%)	698 (100%)	1318 (99.7%)	957 (100%)	1699 (99.4%)
	Yes	0 (0%)	3 (0.3%)	0 (0%)	4 (0.3%)	0 (0%)	10 (0.6%)
EW2							
	No	587 (100%)	958 (99.7%)	698 (100%)	1318 (99.7%)	957 (100%)	1703 (99.6%)
	Yes	0 (0%)	3 (0.3%)	0 (0%)	4 (0.3%)	0 (0%)	6 (0.4%)
EW3							
	No	587 (100%)	960 (99.9%)	698 (100%)	1320 (99.8%)	957 (100%)	1704 (99.7%)
	Yes	0 (0%)	1 (0.1%)	0 (0%)	2 (0.2%)	0 (0%)	5 (0.3%)
Frequency of hypothyroidism[i]							
Time of study							
	No	535 (91.1%)	896 (93.2%)	630 (90.3%)	1253 (94.8%)	906 (94.7%)	1592 (93.2%)
	Yes	52 (8.9%)	65 (6.8%)	68 (9.7%)	69 (5.2%)	51 (5.3%)	117 (6.8%)
EW1							
	No	552 (94.0%)	914 (95.1%)	667 (95.6%)	1289 (97.5%)	927 (96.9%)	1660 (97.1%)
	Yes	35 (6.0%)	47 (4.9%)	31 (4.4%)	33 (2.5%)	30 (3.1%)	49 (2.9%)
EW2							
	No	558 (95.8%)	925 (96.3%)	676 (96.8%)	1297 (98.1%)	937 (97.9%)	1675 (98.0%)
	Yes	29 (4.9%)	36 (3.7%)	22 (3.2%)	25 (1.9%)	20 (2.1%)	34 (2.0%)
EW3							
	No	582 (99.1%)	958 (99.7%)	690 (98.9%)	1317 (99.6%)	953 (99.6%)	1703 (99.6%)
	Yes	5 (0.9%)	3 (0.3%)	8 (1.1%)	5 (0.4%)	4 (0.4%)	6 (0.4%)
Frequency of hyperthyroidism[j]							
Time of study							
	No	569 (96.9%)	945 (98.3%)	667 (95.6%)	1283 (97.1%)	943 (98.5%)	1680 (98.3%)
	Yes	18 (3.1%)	16 (1.7%)	31 (4.4%)	39 (3.0%)	14 (1.5%)	29 (1.7%)
EW1							

		Canada		Italy		Norway	
		Case (N=587)	**Control (N=961)**	**Case (N=698)**	**Control (N=1322)**	**Case (N=957)**	**Control (N=1709)**
	No	575 (98.0%)	947 (98.5%)	678 (97.1%)	1299 (98.3%)	950 (99.3%)	1692 (99.0%)
	Yes	12 (2.0%)	14 (1.5%)	20 (2.9%)	23 (1.7%)	7 (0.7%)	17 (1.0%)
EW2							
	No	576 (98.1%)	947 (98.5%)	685 (98.1%)	1305 (98.7%)	951 (99.4%)	1697 (99.3%)
	Yes	11 (1.9%)	14 (1.5%)	13 (1.9%)	17 (1.3%)	6 (0.6%)	12 (0.7%)
EW3							
	No	586 (99.8%)	961 (100%)	696 (99.7%)	1319 (99.8%)	955 (99.8%)	1700 (99.5%)
	Yes	1 (0.2%)	0 (0%)	2 (0.3%)	3 (0.2%)	2 (0.2%)	9 (0.5%)

a. Number of missing education variables that were imputed: 50 in Canada, 59 in Italy, 42 in Norway.
b. Number of missing ages of RA diagnosis that were imputed: 2 in Canada, 7 in Italy, and 6 in Norway.
c. Number of missing ages of psoriasis diagnosis that were imputed: 3 in Canada, 7 in Italy, and 23 in Norway.
d. Number of missing ages of SLE diagnosis that were imputed: 0 in Canada, 1 in Italy, and 0 in Norway.
e. Number of missing ages of T1D diagnosis that were imputed: 1 in Canada, 4 in Italy, and 2 in Norway.
f. Number of missing ages of CD diagnosis that were imputed: 0 in Canada, 2 in Italy, and 5 in Norway.
g. Number of missing ages of UC diagnosis that were imputed: 2 in Canada, 5 in Italy, and 6 in Norway.
h. Number of missing ages of celiac diagnosis that were imputed: 1 in Canada, 1 in Italy, and 0 in Norway.
i. Number of missing ages of hypothyroidism diagnosis that were imputed: 8 in Canada, 26 in Italy, and 38 in Norway.
j. Number of missing ages of hyperthyroidism diagnosis that were imputed: 3 in Canada, 10 in Italy, and 8 in Norway.

Note: it is possible that some sections do not add exactly to 100%; this is due to rounding discrepancies in R.

The association between having any AiD and the risk of MS

The first analysis, conducted when considering the three aforementioned exposure windows, assessed the association between the presence of any AiD and the subsequent risk of MS. The crude and sex- and age-adjusted results are presented in Table 4.3. A visual representation of these results can be found in the Supplemental Figure 4.2 (Figure S-4.2).

When considering EW1, the age- and sex-adjusted ORs (95% CI) show that, in Canada and Italy, having any AiD was associated with a 1.47 (1.07-2.03)- and 1.36 (1.02-1.82)- times greater risk of MS compared to individuals with no AiDs, respectively. The OR for MS in Norway was 1.00 (0.77-1.30), which is not suggestive of an increased risk of MS. When considering EW2, the risk of MS seen in Canada and Italy increased, with having any AID being associated with a 1.61 (1.13-2.29)- and 1.41 (1.03-1.93)- times greater risk of MS compared to individuals with no AiD, respectively. While the risk also increased in Norway when considering EW2 (1.09 (0.83-1.45)), this continued to not suggest evidence of an increased risk of MS. When considering EW3 focusing on exposure prior to the age of 18, the adjusted ORs for MS were 0.95 (0.53-1.73) in Canada, 1.26 (0.76-2.07) in Italy, and 0.75 (0.48-1.18) in Norway. Therefore, no evidence of an association between having any AiD and an increased risk of MS was observed in any country during the exposure period of childhood or adolescence.

Table 4.3. Crude and adjusted ORs and 95% CIs for the association between having any AiD and MS using various exposure periods.

	EW1: Exposure with no time lag		**EW2: Exposure with 5-year time lag**		**EW3: Childhood or adolescent exposure**	
	Crude OR (95% CI)	Adjusted OR[a] (95% CI)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Canada						
Having any AiD	1.22 (0.90-1.65)	1.47 (1.07-2.03)	1.28 (0.92-1.79)	1.61 (1.13-2.29)	1.24 (0.70-2.20)	0.95 (0.53-1.73)
Italy						
Having any AiD	1.29 (0.97-1.72)	1.36 (1.02-1.82)	1.34 (0.99-1.82)	1.41 (1.03-1.93)	1.24 (0.75-2.04)	1.26 (0.76-2.07)
Norway						
Having any AiD	0.95 (0.73-1.23)	1.00 (0.77-1.30)	1.04 (0.79-1.37)	1.09 (0.83-1.45)	0.75 (0.48-1.18)	0.75 (0.48-1.18)

a. Adjusted for age and sex

The association between individual AiDs and the risk of MS

The second analysis treated each AiD separately. The crude, age- and sex- adjusted, and fully adjusted results are presented in Table 4.4. A visual representation of these results can be found in the Supplemental Figure 4.3 (Figure S-4.3). If fewer than 5 cases or controls had one of the AiDs, the OR and 95% CI were not calculated. As a result, we did not estimate the association for CD, SLE, or celiac disease in any of the countries. For the same reason, the estimates for T1D and RA could not be calculated in Canada, and T1D could also not be calculated in Norway.

When considering EW1, the AiD that showed the highest effect on the risk of MS was hypothyroidism in Canada and Italy. The fully adjusted ORs (age, sex, and past body size) for MS show that hypothyroidism was associated with a 1.92 (1.14-3.23) and 1.93 (1.12-3.32) times greater risk of MS in Canada and Italy, respectively. In Norway, the fully adjusted OR for MS in individuals with hypothyroidism was 1.13 (0.68-1.88). In Canada, the fully adjusted OR (age, sex, parental smoking, smoking history, and past body size) for MS also shows that psoriasis was associated with a 1.86 (1.03-3.37) times greater risk of MS. In Italy and Norway, the fully adjusted ORs for MS in individuals with psoriasis were 1.38 (0.77-2.47) and 1.31 (0.89-1.93), respectively. When considering EW2, the majority of the ORs were similar to the ORs estimated using EW1. Using EW2, hypothyroidism was associated with the strongest risk of MS in Canada at 2.24 (1.25-4.01) times greater risk of MS in individuals with hypothyroidism when adjusting for age, sex, and past body size. For EW2, the associations between hypothyroidism and the risk of MS in Italy and Norway were 1.77 (0.92-3.39) and 1.19 (0.66-2.15), respectively.

Table 4.4. Crude and adjusted ORs and 95% CIs for the association between having each individual AiD and MS using various exposure periods.

	EW1: Exposure with no time lag			EW2: Exposure with 5-year time lag		
	Crude OR (95% CI)	**Adjusted OR[a] (95% CI)**	**Fully Adjusted OR (95% CI)**	**Crude OR (95% CI)**	**Adjusted OR[a] (95% CI)**	**Fully Adjusted OR (95% CI)**
Canada						
Psoriasis	1.30 (0.80-2.11)	1.21 (0.72-2.01)	1.86 (1.03-3.37)[b]	1.19 (0.68-2.08)	1.15 (0.64-2.06)	1.78 (0.92-3.41)[b]
T1D	nc	nc	nc	nc	nc	nc
RA	nc	nc	nc	nc	nc	nc
UC	1.78 (0.63-5.06)	2.32 (0.80-6.76)	n/a	2.40 (0.76-7.59)	3.06 (0.94-9.97)	n/a
Hypothyroidism	1.19 (0.75-1.89)	1.63 (1.00-2.64)	1.92 (1.14- 3.23)[c]	1.25 (0.74-2.10)	1.83 (1.06-3.16)	2.24 (1.25- 4.01)[c]
Hyperthyroidism	1.40 (0.64-3.06)	1.84 (0.81-4.17)	n/a	1.23 (0.55-2.79)	1.68 (0.72-3.91)	n/a
Italy						
Psoriasis	1.25 (0.72-2.15)	1.26 (0.73-2.17)	1.38 (0.77-2.47)[b]	1.26 (0.70-2.26)	1.27 (0.70-2.29)	1.46 (0.78-2.74)[b]
T1D	1.65 (0.74-3.69)	1.72 (0.76-3.86)	1.63 (0.71-3.73)[c]	2.06 (0.88-4.85)	2.14 (0.91-5.03)	1.94 (0.81-4.63)[c]
RA	0.51 (0.24-1.07)	0.52 (0.25-1.10)	0.61 (0.28-1.34)[b]	0.56 (0.25-1.24)	0.57 (0.25-1.27)	0.73 (0.31-1.69)[b]
UC	1.75 (0.77-3.99)	1.84 (0.80-4.22)	n/a	1.80 (0.75-4.32)	1.89 (0.78-4.55)	n/a
Hypothyroidism	1.74 (1.03-2.92)	1.85 (1.10-3.13)	1.93 (1.12-3.32)[c]	1.62 (0.87-3.00)	1.72 (0.92-3.21)	1.77 (0.92-3.39)[c]
Hyperthyroidism	1.59 (0.84-3.03)	1.73 (0.90-3.33)	n/a	1.47 (0.67-3.22)	1.60 (0.73-3.53)	n/a
Norway						
Psoriasis	1.08 (0.76-1.54)	1.12 (0.79-1.60)	1.31 (0.89-1.93)[b]	1.14 (0.79-1.65)	1.19 (0.82-1.71)	1.46 (0.98-2.19)[b]
T1D	nc	nc	nc	nc	nc	nc
RA	0.70 (0.35-1.41)	0.75 (0.37-1.51)	0.88 (0.42-1.83)[b]	0.72 (0.34-1.52)	0.77 (0.36-1.65)	0.90 (0.41-1.99)[b]
UC	0.75 (0.26-2.16)	0.80 (0.28-2.32)	n/a	0.95 (0.32-2.86)	1.03 (0.34-3.11)	n/a
Hypothyroidism	1.02 (0.63-1.65)	1.12 (0.69-1.82)	1.13 (0.68-1.88)[c]	1.08 (0.62-1.91)	1.19 (0.67-2.10)	1.19 (0.66-2.15)[c]
Hyperthyroidism	0.71 (0.27-1.87)	0.77 (0.29-2.03)	n/a	0.75 (0.23-2.44)	0.82 (0.25-2.65)	n/a

a. Adjusted for age and sex
b. Adjusted for age, sex, parental smoking, smoking history, and past body size
c. Adjusted for age, sex, and past body size
nc = not calculated
n/a = not applicable (no additional confounders)

4.4 Discussion

In our multicenter study, we found evidence that having any of our nine AiDs is associated with an increased risk of MS in Canada and Italy using an exposure period with no time lag and with a five-year time lag between the diagnosis of the AiD and MS. The increased risk of MS was not observed when restricting to childhood and adolescent AiD exposure. This finding may be attributable to the small sample size in this analysis due to the low incidence of several AiDs in individuals less than 18 years of age. No association between having any AiD and the risk of MS was observed in Norway when considering any of the exposure windows.

When looking at the individual AiD models, we found evidence in Canada that psoriasis is associated with an increased risk of MS in the fully adjusted model when considering EW1. In

previous published literature, it was found by Egeberg et al. that mild and severe psoriasis may be associated with an increased risk of MS (10). Despite this, four other studies noted no increased risk of MS associated with psoriasis (6-9). Additionally, we found evidence in Canada that having hypothyroidism is associated with an increased risk of MS both when considering EW1 with no time lag and EW2 with a five-year time lag when adjusting for age, sex, and past body size. In Italy, hypothyroidism was associated with an increased risk of MS when considering EW1. No association between hypothyroidism and the risk of MS was observed in Norway under any of the three exposure windows. This may suggest a distinct combination of genetic or environmental factors at play which may be modifying the association between AiDs and MS in Norway. It should be noted that the number of missing diagnosis ages for hypothyroidism was highest in Norway compared to Canada and Italy; of the 165 people with hypothyroidism in the Norwegian sample, 23% did not provide any age of diagnosis, compared to 16% in Italy and 6.8% in Canada. These ages were imputed using PMM. The larger percentage of missing data in Norway could affect the estimate and cause a bias (of unknown direction) in the association between hypothyroidism and the increased risk of MS if the imputed ages resulted in a larger proportion of people to be misclassified as either exposed or unexposed.

Past research on the association between hypothyroidism and the risk of MS has yielded conflicting results; Maroufi et al. found that hypothyroidism was linked to an increased risk of the primary-progressive MS (PPMS) disease course (7). While this is an interesting finding, it is difficult to compare to our own results since PPMS makes up only ~15% of all disease courses of MS (35). Four other studies looking at the association between hypothyroidism (or Hashimoto's thyroiditis, one of the most common causes of hypothyroidism) and the risk of MS found no link (6, 8, 9, 12). Perga et al. (2018) sought to investigate the common molecular

mechanisms between MS and Hashimoto's thyroiditis and uncover common genetic susceptibility and environmental exposures (36); they found that MS and Hashimoto's thyroiditis share some common deregulated anti-inflammatory mechanisms through the BACH2/PDCD5-FOXP3 pathways and Tregs, which are involved in a variety of AiDs (36). There is a need for further research on the common molecular mechanisms or potential environmental triggers between hypothyroidism and MS.

Strengths and Risk of Bias

One data limitation that we encountered was that the prevalence of some AiDs remained too low for the individual analyses in one or more of the countries. This is particularly a limitation for T1D, CD, and SLE, which had been previously linked with an increased risk of MS in past literature (9, 11, 14). As with any retrospective study, we are limited by the recall ability of participants and run the risk of participants not accurately reporting their own exposures. This risk was mitigated in EnvIMS by allowing participants to enlist the help of friends and family members in filling out the questionnaires. Using identical mailed questionnaires for cases and controls removes the likelihood of interviewer bias found in some of the previous case-control studies conducted on the link between AiDs and MS, whereby interviewers were either not blinded to participant status or different interview methodologies were used to question cases versus controls (6, 7). We are additionally limited by the response rate of EnvIMS. Differential response rate can lead to bias if those who did not respond were different from responders in factors related to our AiD exposures. This would particularly be an issue if the cases who had additional AiDs were less likely to respond to the questionnaires due to increased impairment, which would lead us to underestimate the association between AiDs and MS. Residual confounding is also a potential source of bias, particularly in the first method

of analysis which combines all AiDs as one variable. Adjusting only for sex and age in these models may fail to account for other confounding factors, which could lead to a spurious association between having an AiD and the increased risk of MS.

An important strength of our analysis derives from using data from the EnvIMS study. Case ascertainment in EnvIMS was thorough and involved recruitment from MS databases and neurology clinics, allowing us to be confident that cases were truly diagnosed with MS. Cross-checking MS databases, as well as the self-report of MS, also allowed us to exclude controls which have MS. Additionally, having the reported ages of diagnosis for each AiD and MS allowed us to establish temporality between the illnesses to ensure that the AiDs truly preceded MS. By introducing a five-year time lag exposure window into our analyses, we increased the level of certainty that the AiDs were diagnosed prior to MS. We clearly defined the exposure periods in controls using index ages to ensure they were comparable to the cases. Several past case-control studies on the association between AiDs and the risk of MS failed to describe how they considered the exposure period in controls (7, 8, 12). Additionally, our current study is not only the first multinational study on the association between AiDs and the risk of MS, but also includes the highest number of cases from all previous case-control studies conducted on this association (6-9, 12). By using EnvIMS, we were able to conduct our analyses in three unique countries while knowing that all participant recruitment and data collection was done with consistent methodologies across the three countries. By removing any inconsistency in methodologies that are usually present when comparing results from studies conducted in different countries, we are able to highlight the differing associations observed between AiDs and MS across the three countries.

4.5 Conclusion

The current study is the first to use common methodologies across several countries to examine the association between AiDs and the risk of MS. Our differing results across the countries suggest unique relationships may be at play, particularly in Norway. Our work improves on previous case-control studies due to our large sample size, common methodology across participants and countries, our reduced risk of interviewer bias, and our ability to control for confounders. We found evidence in Canada and Italy that having any AiD is associated with an increased risk of MS both when no time lag and a five-year time lag are considered between the diagnosis of the AiDs and MS. Furthermore, we found compelling evidence that hypothyroidism is associated with an increased risk of MS in Canada (both with no time lag and a five-year time lag) and in Italy (no time lag), but not in Norway. We additionally saw an association between psoriasis and an increased risk of MS in Canada when no time lag was included in our model. Based on these findings, it would be beneficial to further explore the association between hypothyroidism and MS through molecular and genetic studies. While no evidence of an association between the other AiDs and the risk of MS was found, several of the AiDs that had previously been associated with an increased risk of MS, particularly T1D, CD, and SLE, could not be individually analyzed due to lack of data. Further research on how these AiDs modulate the risk of MS should be conducted.

4.6 References

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4.7 Supplemental

Assigning Index Ages

We let n = number of cases, m = number of controls, and e = ⌊m/n⌋, where e is the integer part of m/n. The index age of cases was set as the age at which they were diagnosed with MS. Cases and controls were separately ordered from youngest to oldest based on age at time of study. Next, e*n of the youngest controls was sampled, and the index ages of the youngest cases were assigned to these ordered controls, where each case's age was assigned to e controls. Subsequently, a random sample of e*n was taken from the remaining unassigned controls and was ordered youngest to oldest and assigned the index ages of the ordered cases. This sampling process was continued until there were no longer enough remaining unassigned controls to sample e*n controls. The remaining controls were then assigned an index age from a case who had the same current age or was younger. To account for the older controls in the Canadian dataset, only cases older than 50 years were used to assign index ages after the first round of sampling, therefore ensuring that the older controls would be assigned index ages from the cases closest to them in age.

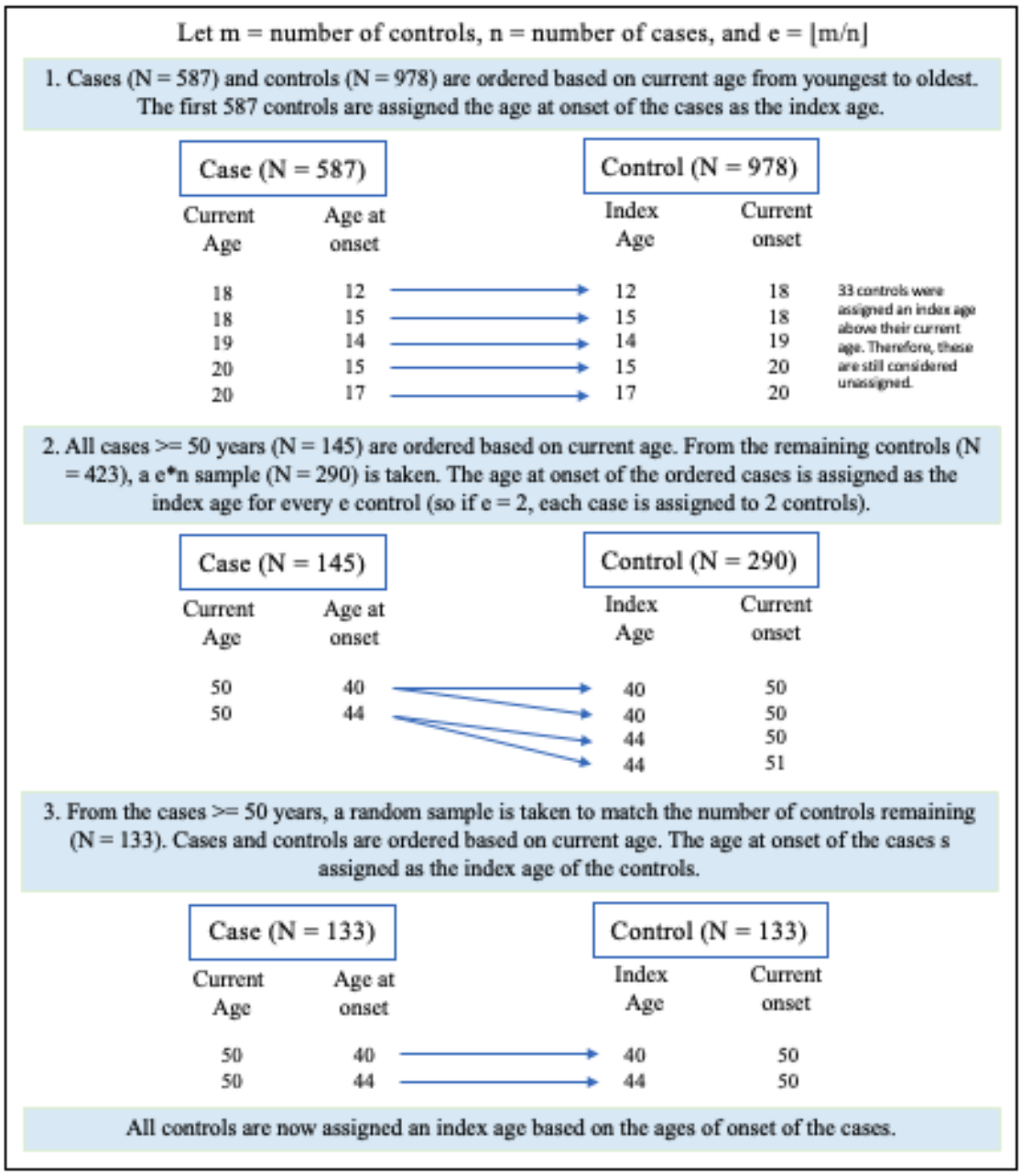

Figure S-4.1. Example of how index ages were assigned to controls in the Canadian EnvIMS study

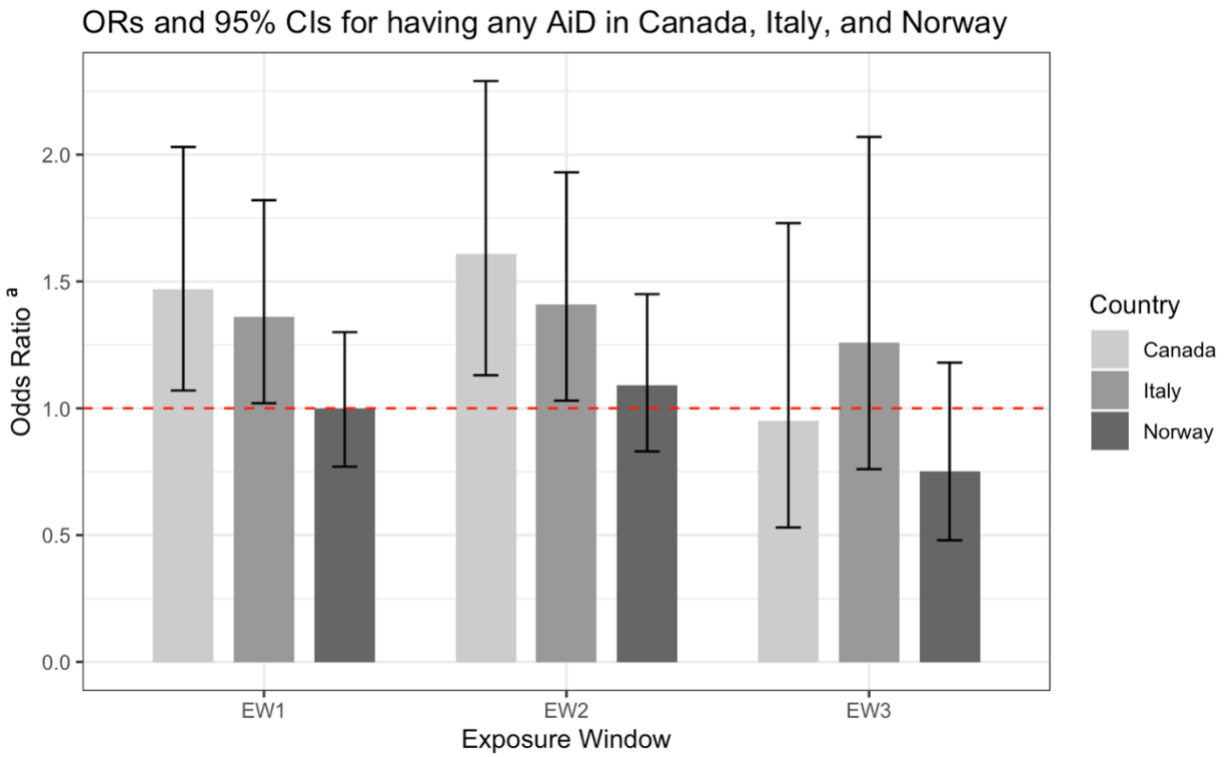

Figure S-4.2. The odds ratios and 95% confidence intervals for the association between having any AiD and the risk of MS in Canada, Italy, and Norway when considering the three defined exposure windows.

a. adjusted for age and sex

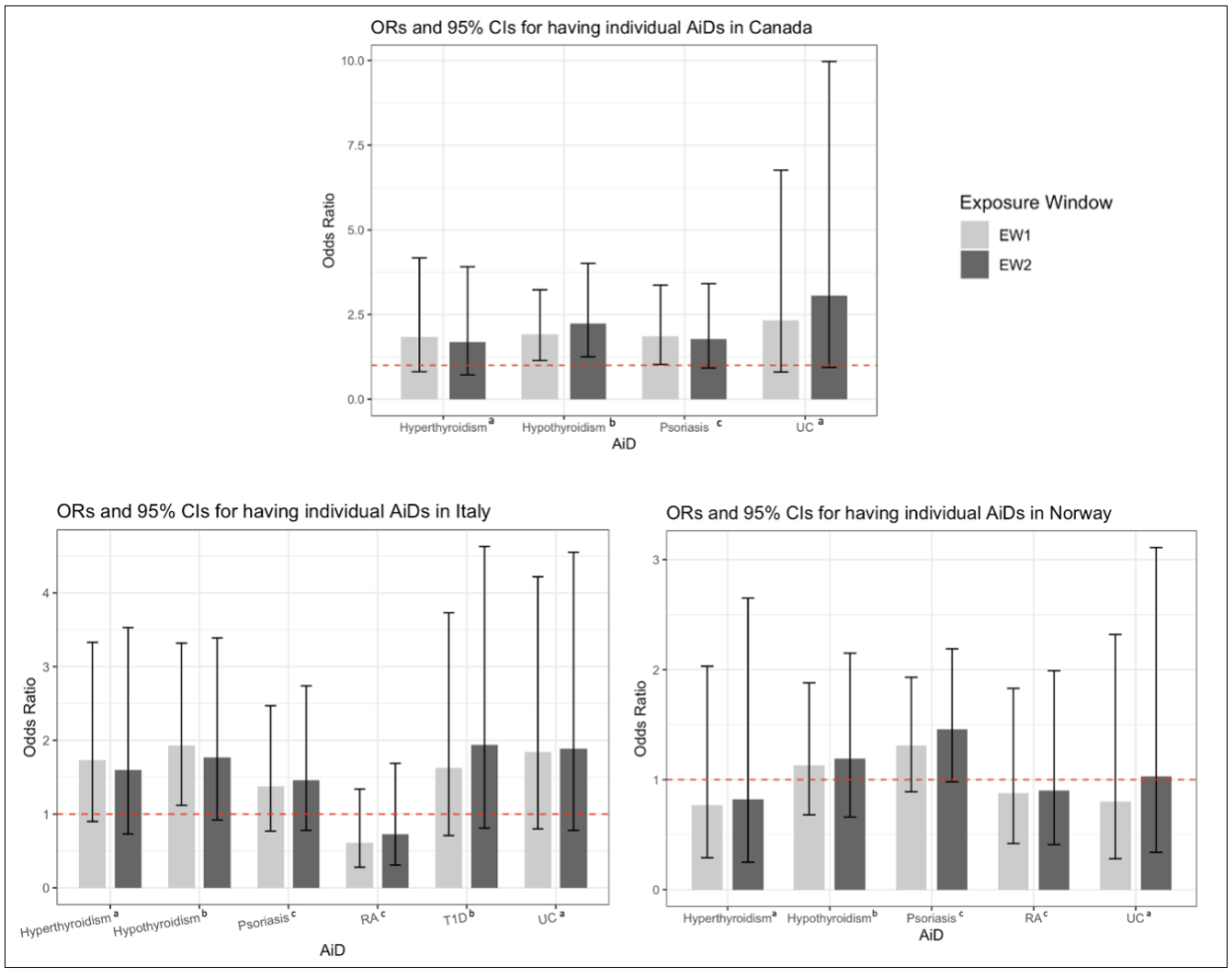

Figure S-4.3. The odds ratios and 95% confidence intervals for the association between each AiD and the risk of MS in Canada, Italy, and Norway when considering the two defined exposure windows.

a. adjusted for age and sex
b. adjusted for age, sex, and past body size
c. adjusted for age, sex, parental smoking, smoking history, and past body size

Chapter 5: Discussion of Findings and Overall Conclusion

This chapter discusses the findings and implications of the analysis presented in Chapter 4 on the association between AiDs and the risk of MS, as well as how these results compare to existing research, and possible mechanisms that may explain the associations found.

5.1 Findings and comparison to past research

While several risk factors, including genetic, infectious, and environmental factors, have been identified for MS, the cause of MS remains unknown. The goals of this thesis were to summarize the existing knowledge on the relationship between AiDs and the risk of MS and to explore the association between having one or more of nine AiDs of interest (RA, T1D, psoriasis, CD, UC, SLE, celiac disease, hypothyroidism, and hyperthyroidism) and the subsequent risk of MS when considering three exposure windows. Logistic regression was used to estimate the crude, age- and sex-adjusted, and fully adjusted ORs and 95% CIs for the association between the AiDs and MS.

5.1.1 Having any AiD and the risk of MS

In our analysis, we found that having any of the nine AiDs was associated with an increased risk of MS both when considering EW1 and EW2 in Canada (1.47 (1.07-2.03) and 1.61 (1.13-2.29), respectively) and Italy (1.36 (1.02-1.82) and 1.41 (1.03-1.93), respectively). This association was not seen in Norway. In all three countries, the exposure of having any of the nine AiDs during the period of childhood and adolescence was not associated with an increased risk of MS. In one study previously discussed in Chapter 2, Zorzon et al. found that having an AiD prior to MS was associated with an increased risk of MS of 10.8 (2.5-46.8) (62). This study, however, does not elaborate on which AiDs were reported within their cohort and therefore our results cannot be compared to theirs. Overall, from our analysis, we determined that having any

of the nine AiDs any time prior to MS and at least five years prior to MS is associated with an increased risk of MS in Canada and Italy. While the period of childhood and adolescence has previously been found to be an important exposure window for many MS risk factors, such as IM, obesity, and vitamin D deficiency, we did not find evidence of a higher risk of MS associated with having any of the nine AiDs when restricting to this period (43).

5.1.2 Individual AiDs and the risk of MS

We review our results on the association between each AiD and the risk of MS and compare our findings to the past research described in Chapter 2. Due to insufficient sample sizes, we did not estimate the association for CD, SLE, or celiac disease in any country. Furthermore, we were unable to calculate estimates for T1D and RA in Canada and T1D in Norway for the same reason.

Psoriasis

We found evidence of an association between psoriasis and an increased risk of MS in Canada in the period any time preceding MS when adjusting for age, sex, parental smoking, smoking history, and past body size (1.86 (1.03-3.37)). This association was not apparent when we introduced a five-year time lag between the diagnosis of psoriasis and MS. We did not find any evidence of an association between psoriasis and MS in Italy or Norway in any of the exposure windows considered. In past research, Egeberg et al. found that individuals with mild and severe psoriasis were both at an increased risk of MS compared to individuals who did not have psoriasis (IRR = 1.84 (1.46-2.30) and IRR = 2.61 (1.44-4.74), respectively) (12). Four other studies which looked at the link between psoriasis and MS found no association (11, 13, 15, 16).

Type-1 Diabetes

An estimate for the association between T1D and MS could only be calculated in Italy; no increased risk of MS was found when considering any of the exposure windows. In the published literature, six studies looked at the association between T1D and MS. Of note, Nielsen et al. found a three-fold increase in the observed number of MS cases in their T1D cohort across follow-up compared to the expected number of cases (RR = 3.26 (1.80-5.88)) (17). Magyari et al. additionally reported an increased risk of MS associated with T1D in males (OR = 3.34 (1.40-7.02)) (15). Meanwhile, Abbasi et al. reported a decreased risk of MS associated with T1D (OR = 0.11 (0.01-0.99)) (10). In three other studies which assessed the link between T1D and the risk of MS, none found any association (11, 13, 16).

Ulcerative Colitis

Across all three countries, we found no evidence that UC is associated with the risk of MS. In the literature examined in Chapter 2, none of the four studies which assessed the association between UC and the risk of MS found an association (11, 15, 16, 18). Two studies looked at the association between IBD and the risk of MS (10, 13); Langer-Gould et al. found that IBD was associated with an increased risk of MS (OR = 2.7 (1.1-6.8)), however, the definition of IBD includes both UC and CD and we cannot conclude that this is evidence that UC may increase the risk of MS (13). Since cases of IBD have been shown to be evenly distributed between CD and UC, it is difficult to discriminate between these two AiDs when considering the umbrella term of IBD (66).

Hypothyroidism

In our analysis, we found evidence of an association between hypothyroidism and MS when considering the exposure period any time prior to MS in Canada (1.92 (1.14-3.32)) and Italy (1.93 (1.12-3.32)) when adjusting for age, sex, and past body size. We additionally found

evidence of a link between hypothyroidism and the increased risk of MS in Canada when a five-year time lag period is considered between the diagnosis of both diseases in Canada (2.24 (1.25-4.01)). We did not find any evidence of this association in Norway using any of the exposure periods. In past literature, five studies looked at the risk of MS associated with either hypothyroidism or Hashimoto's thyroiditis; Hashimoto's thyroiditis is an autoimmune condition and is the most common cause of hypothyroidism in developed countries (74). It is important to note that not all causes of hypothyroidism are autoimmune; other potential causes of hypothyroidism include iodine deficiency, pituitary gland dysfunction, and resistance to thyroid-stimulating hormone (75). Maroufi et al. found that hypothyroidism was associated with an increased risk of PPMS (OR = 3.20 (1.23-8.30)) (16). While this is an interesting finding, the PPMS disease course represents only ~10-15% of all cases of MS diagnosis. Therefore, it is difficult to compare this result to ours and conclude that they are similar findings. Of the other studies that looked at this relationship, none found an association between hypothyroidism (11) or Hashimoto's thyroiditis (10, 13, 15) and MS.

Hyperthyroidism

Across all three countries, we found no evidence that hyperthyroidism is associated with the risk of MS. There were four previous studies that explored the association between hyperthyroidism or Graves' disease and the risk of MS; it has been demonstrated that Graves' disease is the most common cause of hyperthyroidism worldwide (76). Other potential causes of hyperthyroidism include high exposure to iodine, inappropriate secretion of thyroid-stimulating hormone, and excess intake of thyroid hormone (75). Of the studies that looked at this relationship, none found an association between hyperthyroidism (11, 16) or Graves' disease (13, 15) and MS.

Overall, based on the magnitude of the ORs and 95% CIs in Canada and Italy, there is evidence in our study that hypothyroidism confers an increased risk of MS when the period of exposure is set to any time preceding the diagnosis of MS. The Canadian estimates show evidence that hypothyroidism may also confer an increased risk of MS when the diagnosis of hypothyroidism is required to precede that of MS by at least five years. Specifically in Canada, reducing the exposure period from any time before MS to at least five years prior to MS diagnosis increases the magnitude of the estimated risk for MS associated with hypothyroidism. When a larger exposure window is considered, there is a risk of accidentally capturing irrelevant exposures in our analysis which may attenuate the risk estimates of MS. We additionally see in our analysis of having any of the nine AiDs that when the exposure period is narrowed to at least five years prior to MS, the magnitude of our estimates increases in both Canada and Italy. Introducing a lag period after the diagnosis of the AiD helps to not only be more confident that the AiD precedes MS, but also reduces the risk of including noise in our analysis which may conceal the true association between the AiDs and MS. Interestingly, in Italy the estimated risk for MS associated with hypothyroidism decreased when considering the exposure window with a five year time lag compared to the exposure any time prior to MS. This is a deviation from the general trend of increasing magnitudes that is observed in the other models when the five-year time lag is imposed.

It is important to note that the term “hypothyroidism” includes both the autoimmune cause of hypothyroidism and the non-autoimmune causes. While the majority of hypothyroidism is caused by Hashimoto’s thyroiditis, it is possible that some of the individuals who reported having hypothyroidism did not have an autoimmune cause. In this case, we run the chance that

the association seen between hypothyroidism and the increased risk of MS may actually be driven by people who have non-autoimmune hypothyroidism. There have not been many studies attempting to explore a possible common pathological mechanism between hypothyroidism or Hashimoto thyroiditis and MS. Perga et al. (2018) investigated the mechanisms which could lead to the frequent coexistence between MS and Hashimoto's thyroiditis and found there could plausibly exist a common dysregulated mechanism shared by the two AiDs through the BACH2/PDCD5-FOXP3 pathways and regulatory T cells (77).

5.2 Limitations

Several steps were undertaken during the planning of the EnvIMS study to minimize the risk of bias. Despite this, there remains a number of potential sources of bias that may have affected the results presented in this thesis, several of which are intrinsic to case-control study designs.

5.2.1 Recall Bias

Recall bias is an intrinsic issue in all case-control studies whereby disease status may influence participants' recall of exposures (63). If disease status causes cases to overestimate their exposures compared to controls, this may lead to differential misclassification of exposure and overestimate the apparent role of AiDs on the risk of MS. A study assessing the accuracy of self-reporting of AiDs compared to electronic medical records found that conditions such as Hashimoto's thyroiditis, T1D, and RA had positive predictive values lower than 50% (meaning less than 50% of self-report diagnoses were supported by the medical records) (78). They also found that some people did not self-report having psoriasis despite the illness being indicated in their medical records; this was mostly the case for individuals who had concurrent diagnosis for psoriasis and another dermatological condition (78). Previous research on the validation of self-

report questionnaires for the reporting of comorbidities in individuals with MS found that in general, the frequency of comorbidities was higher based on the questionnaires than medical records (79). They found that agreement was higher for conditions that were well-defined and require ongoing care, such as diabetes, but lower for less clearly defined conditions, such as arthritis (79). However, the authors note that frequency of AiDs was too low in their study population for meaningful interpretation. Of note, the EnvIMS-Q was tested for reliability and was shown to have substantial agreement on most sections, including medical history (68).

One step taken in the EnvIMS-Q in order to minimize recall bias was to give identical questionnaires to cases and controls, which means that cases were not prompted more than controls to recall their exposures. It has been proposed that when collecting information regarding exposures that may vary over time (i.e., smoking history), recall can be increased by posing questions in a way that forces individuals to refer to a specific period of time in their lives (80). In EnvIMS, questions on potential confounders, such as smoking history and past body size, were asked in a way that individuals had to consider specific periods of their lives (i.e., frequency of smoking at the age of 21-25 years) in order to facilitate recall. Additionally, better recall in EnvIMS was further encouraged by allowing participants to receive help from family to complete the questionnaire.

5.2.2 Response Rate

The percentage of responses in EnvIMS was lower in controls than cases. The response percentage in EnvIMS in cases and controls, respectively, were 83% and 59% in Canada, 43% and 21% in Italy, and 70% and 36% in Norway. In a situation of differential response, non-response bias may occur if those who did not respond are different from the responders in factors related to our AiD exposures. Non-response bias can occur if people who agreed to participate

were healthier than those who did not. This may be particularly true if cases who had additional AiDs were less likely to respond to the questionnaires due to increased impairment. If cases with AiDs were less likely to respond than controls, this would lead to an underestimation of the association between AiDs and the risk of MS.

5.2.3 Data limitations

Based on existing literature, we initially hypothesized that T1D, CD, SLE, psoriasis, and hypothyroidism could be associated with an increased risk of MS. However, a low prevalence of some of the AiDs in our study meant that not all individual associations could be estimated in every country. We decided that if fewer than 5 cases or controls had one of the AiDs, the OR and 95% CI were not calculated. As a result, the associations between CD, SLE, or celiac diseases and MS were not estimated in any of the countries, the association between T1D and MS was not calculated in Canada and Norway, and RA could not be calculated in Canada. To illustrate this challenge with sample sizes, Table 5.1, below, shows the prevalence of the AiDs that were not estimated in each country. This table shows how many people were diagnosed with the AiDs at any time prior to MS.

Table 5.1. Prevalence of the AiDs for which an association with MS was not estimated in some or all countries when considering EW1 (and therefore not estimated when considering EW2 or EW3).

	Canada		**Italy**		**Norway**	
	Case (N=587)	**Control (N=961)**	**Case (N=698)**	**Control (N=1322)**	**Case (N=957)**	**Control (N=1709)**
Frequency of RA						
No	583 (99.3%)	952 (99.1%)	688 (98.6%)	1288 (97.4%)	945 (98.7%)	1680 (98.3%)
Yes	**4 (0.7%)**	9 (0.9%)	10 (1.4%)	34 (2.6%)	12 (1.3%)	29 (1.7%)
Frequency of SLE						
No	586 (99.8%)	960 (99.9%)	698 (100%)	1319 (99.8%)	1709 (100%)	957 (100%)
Yes	**1 (0.2%)**	**1 (0.1%)**	**0 (0%)**	**3 (0.2%)**	**0 (0%)**	**0 (0%)**
Frequency of T1D						
No	584 (99.5%)	959 (99.8%)	686 (98.3%)	1309 (99.0%)	953 (99.6%)	1700 (99.5%)
Yes	**3 (0.5%)**	**2 (0.2%)**	12 (1.7%)	13 (1.0%)	**4 (0.4%)**	9 (0.5%)
Frequency of CD						
No	584 (99.5%)	958 (99.7%)	696 (99.7%)	1318 (99.7%)	952 (99.5%)	1706 (99.8%)
Yes	**3 (0.5%)**	**3 (0.3%)**	**2 (0.3%)**	**4 (0.3%)**	5 (0.5%)	**3 (0.2%)**

Frequency of celiac disease						
No	587 (100%)	958 (99.7%)	698 (100%)	1318 (99.7%)	957 (100%)	1699 (99.4%)
Yes	**0 (0%)**	**3 (0.3%)**	**0 (0%)**	**4 (0.3%)**	**0 (0%)**	10 (0.6%)

5.2.4 Residual Confounding

As in most observational studies, there is the potential for residual confounding through unmeasured confounders or unadjusted variables, particularly in the first models where all AiDs are grouped as one variable. These models were only adjusted for age and sex, leaving room for unaddressed confounding between the association of one or more AiDs and the risk of MS. Since many AiDs had different confounders, it would be difficult to choose an appropriate set of confounders to adjust for in an analysis which includes any of the nine AiDs as the exposure variable. For example, the confounders considered for the analysis of the association between psoriasis and the risk of MS are age, sex, parental smoking, smoking history, and past body size, whereas the confounders considered in the analysis of UC and the risk of MS are only age and sex. Adjusting for covariates that are not supported as confounders in the association between UC and MS (e.g. parental smoking, smoking history, and body size) may result in reduced precision. Additionally, residual confounding could remain in our adjusted associations for the individual AiD models since several confounders were introduced into the models as grouped, not continuous variables (e.g. smoking history from ages 11-15 years). This residual confounding arises from the fact that variables grouped into a category may still have variability within each level that is not being accounted for in the analysis and therefore does not fully adjust for the effect of the confounder (81). Within each age group, there is additional grouping of response categories which also increases the variability within each level that is not being accounted for in analysis (e.g. 1-4 cigarettes per day, 5-10 cigarettes per day, 11-20 cigarettes per day, 21+ cigarettes per day). Becher (1992) demonstrates, through an example using the

smoking, that categorization of a confounder can yield higher residual confounding compared to the use of the continuous form of the variable (81).

5.2 Strengths

Despite our limitations, this study possesses several strengths which allowed us to improve upon past research looking at the association between AiDs and the risk of MS.

5.2.1 Ascertainment of Cases and Controls

One important strength of this study was in the care taken in the ascertainment of cases and controls. Cases were recruited either from MS registries or MS/neurology clinics, which allowed us to be confident that cases were truly diagnosed with MS according to validated and widely accepted MS criteria. Controls were also cross-checked for self-report of MS in their questionnaires, which allowed us to rule out MS in controls at the time of the study. While other case-control studies on these associations also used validated MS criteria to select cases, most did not report if and/or how they determined that the controls did not have MS (10, 11, 13, 16).

5.2.2 Ascertainment of AiDs and Exposure Periods

The EnvIMS-Q asked participants to report whether they had one of the nine AiDs of interest and also asked for age of diagnosis. Thus, the timing of the diagnosis relative to the diagnosis of MS could be clarified. The EnvIMS-Q was carefully developed and tested for feasibility and reliability across the various countries of EnvIMS, and it was shown that all sections, including the medical history module, had substantial agreement for intraparticipant consistency of answers (68). We additionally improve on past studies by having clearly defined exposure periods for controls which are comparable to that of the cases and were calculated using an index age algorithm proposed by Erin Lundy in her MSc Thesis (82). In past case-

control studies on the association between AiDs and the risk of MS, several failed to specify the considered exposure period in controls (10, 13, 16).

5.2.3 Comparability Across Three Countries

A final important strength of this thesis is that using data from the EnvIMS study allowed us to conduct our analysis in three countries (Canada, Italy, and Norway) using consistent methodologies. Participant recruitment and data collection were conducted with almost identical methods and the EnvIMS-Q was formally assessed for cross-cultural acceptability, feasibility, and reliability. Thus we believe that our results are highly comparable to one another across countries in a way that has not previously been achieved with existing studies on the association between AiDs and the risk of MS. Indeed there are few, if any, case control studies of MS that have been conducted in different countries using the same methodology. It is of note that since the first publications of studies using the EnvIMS data, several other authors have used the EnvIMS-Q (translated into different languages) to permit comparability with the EnvIMS results (83, 84). In addition, the EnvIMS study has been used as a basis for a recent study of Neuromyelitis Optica (NMO) utilizing not only the EnvIMS-Q but also the controls in the Canadian arm of EnvIMS as a comparison to cases of NMO (85).

5.4 Conclusion

This study was, to our knowledge, the first work to use common methodologies across several countries to examine the association between AiDs and the risk of MS. We were in a unique position to assess the differences in the association between AiDs and the risk of MS across three countries with differing environmental exposures and populations. We found evidence, notably in Canada and Italy, that having any one or more of rheumatoid arthritis, psoriasis, systemic lupus erythematosus, type-1 diabetes, Crohn's disease, ulcerative colitis,

celiac disease, hypothyroidism, or hyperthyroidism is associated with an increased risk of MS. This was observed when considering no time lag between the diagnosis of the AiD and MS and also when enforcing a five-year time lag between the diagnosis of the AiD and MS. We also found evidence that hypothyroidism may be associated with an increased risk of MS. This was observed both when considering an exposure of no time lag (Canada and Italy) and a five-year time lag exposure (Canada). Our results are consistent with the notion that there may be a common biological pathway or environmental risk factor between hypothyroidism and MS, or that hypothyroidism may cause immune alterations which increase the likelihood of MS. Our current study was a first step in assessing the association between AiDs and the risk of MS. It is important to remember that in this study, the definition of hypothyroidism may have led to the inclusion of people who have hypothyroidism from non-autoimmune causes. As a future direction, we recommend further research in the association between Hashimoto's thyroiditis (the autoimmune cause of hypothyroidism) and the risk of MS, particularly in the field of genetic and molecular studies. This would help to elucidate whether the association observed in this study between hypothyroidism and MS is indeed due to autoimmune causes. We additionally recommend that a large cohort study be conducted through linkage across several countries to assess the association between a larger number of AiDs and the risk of MS. We have shown in our current analysis that cross-country comparisons are an important consideration on the link between AiDs and MS since some country populations may show a stronger association than others.

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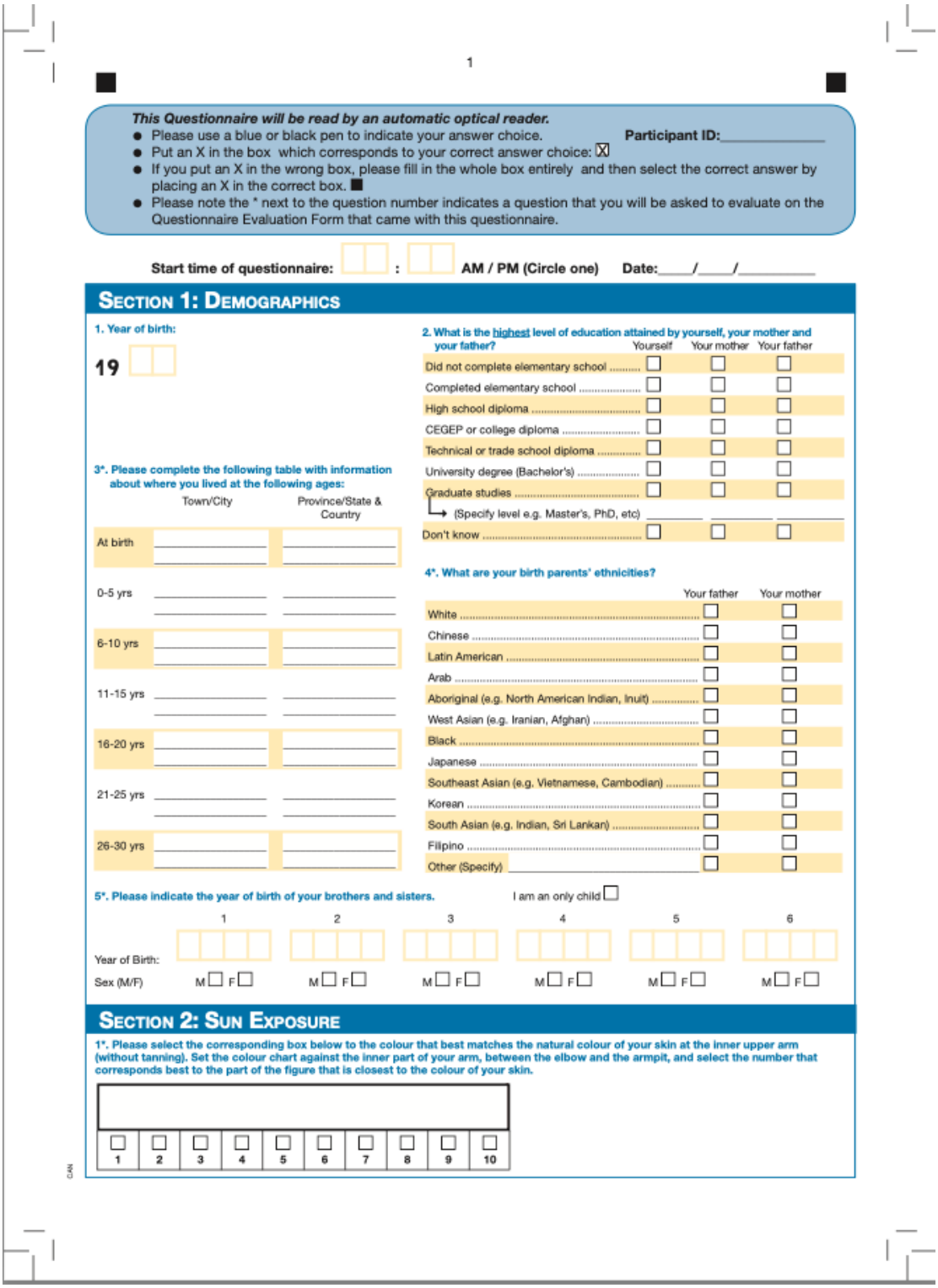
Appendix

Figure A1: The EnvIMS Questionnaire



1

This Questionnaire will be read by an automatic optical reader.
- Please use a blue or black pen to indicate your answer choice.
- Put an X in the box which corresponds to your correct answer choice: ☒
- If you put an X in the wrong box, please fill in the whole box entirely and then select the correct answer by placing an X in the correct box. ■
- Please note the * next to the question number indicates a question that you will be asked to evaluate on the Questionnaire Evaluation Form that came with this questionnaire.

Participant ID:_____

Start time of questionnaire: ☐☐ : ☐☐ **AM / PM (Circle one)** **Date:**____/____/_____

SECTION 1: DEMOGRAPHICS

1. Year of birth:

19 ☐☐

2. What is the highest level of education attained by yourself, your mother and your father?

	Yourself	Your mother	Your father
Did not complete elementary school	☐	☐	☐
Completed elementary school	☐	☐	☐
High school diploma	☐	☐	☐
CEGEP or college diploma	☐	☐	☐
Technical or trade school diploma	☐	☐	☐
University degree (Bachelor's)	☐	☐	☐
Graduate studies	☐	☐	☐
↳ (Specify level e.g. Master's, PhD, etc)	_____	_____	_____
Don't know	☐	☐	☐

3*. Please complete the following table with information about where you lived at the following ages:

	Town/City	Province/State & Country
At birth		
0-5 yrs		
6-10 yrs		
11-15 yrs		
16-20 yrs		
21-25 yrs		
26-30 yrs		

4*. What are your birth parents' ethnicities?

	Your father	Your mother
White	☐	☐
Chinese	☐	☐
Latin American	☐	☐
Arab	☐	☐
Aboriginal (e.g. North American Indian, Inuit)	☐	☐
West Asian (e.g. Iranian, Afghan)	☐	☐
Black	☐	☐
Japanese	☐	☐
Southeast Asian (e.g. Vietnamese, Cambodian)	☐	☐
Korean	☐	☐
South Asian (e.g. Indian, Sri Lankan)	☐	☐
Filipino	☐	☐
Other (Specify) _____	☐	☐

5*. Please indicate the year of birth of your brothers and sisters. I am an only child ☐

	1	2	3	4	5	6
Year of Birth:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
Sex (M/F)	M☐ F☐	M☐ F☐	M☐ F☐	M☐ F☐	M☐ F☐	M☐ F☐

SECTION 2: SUN EXPOSURE

1*. Please select the corresponding box below to the colour that best matches the natural colour of your skin at the inner upper arm (without tanning). Set the colour chart against the inner part of your arm, between the elbow and the armpit, and select the number that corresponds best to the part of the figure that is closest to the colour of your skin.

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

CAN

2

2*. What is the tanning reaction of your skin to its first sun exposure in the summer, with *no* use of sunscreen?

1. Always burn, never tan ☐
2. Usually burn, tan less than average (with difficulty) ☐
3. Sometimes mild burn, tan about average ☐
4. Rarely burn, tan more than average (with ease) ☐
5. Don't know ☐

3*. What is the natural colour of your hair as an adult?

1. Black ☐
2. Dark Brown ☐
3. Light Brown ☐
4. Blonde ☐
5. Red ☐

4*. What colour are your eyes?

1. Black ☐
2. Brown ☐
3. Grey, green ☐
4. Blue ☐
5. Hazel ☐

5*. In the past, in summer, how often did your activities (playing, participating in sports, watching sports, gardening, walking, work activities, etc.) take you outside at the following ages?

	Not that often	Reasonably often	Quite often	Virtually all the time
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐
In the past 3 years	☐	☐	☐	☐

6*. In the past, in winter, how often did your activities (playing, participating in sports, watching sports, shovelling snow, walking, work activities, etc.) take you outside at the following ages?

	Not that often	Reasonably often	Quite often	Virtually all the time
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐
In the past 3 years	☐	☐	☐	☐

7*. At the following ages, where have your work and occupational activities (including parenting, caregiving, etc.) been carried out:

	Mainly indoors	Mainly outdoors	Same time spent indoors and outdoors
16-20 yrs	☐	☐	☐
21-25 yrs	☐	☐	☐
26-30 yrs	☐	☐	☐

8*. On weekends and holidays, how much time did you normally spend outside at these ages:

	Never	<1 hour/day	1-2 hours/day	3-4 hours/day	>4 hours/day
0-5 yrs	☐	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐	☐

9*. How often did you go on vacation to sunny places during winter months at these ages?

	Never/seldom	1 week/year or less	1-2 weeks/year	4+ weeks/year
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐

10*. How often did you use sun protection (sunscreen or protective clothing such as hats, long sleeves) at these ages?

	Never/seldom	Sometimes	Quite often	Almost always
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐

11. How often did you use sunlamps or tanning beds at these ages?

	Never/Seldom	Less than once/year	Less than once/month	Once or more/month
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐

SECTION 3: DIET

We would like to ask you information about your diet when you were a **"teenager" (between 13 and 19 years old).** If your diet changed substantially during this period of time, please try to report the average consumption for the period.

1*. Please indicate in which season(s) you generally consumed the following foods while you were a teenager (age 13-19 years)?
(you may choose more than one checkbox per row).

	Winter	Spring	Summer	Fall	Never/ seldom
Cows' milk (liquid or reconstituted powdered)	☐	☐	☐	☐	☐
Other type of milk (Specify: _____)	☐	☐	☐	☐	☐
Yogurt	☐	☐	☐	☐	☐
Eggs (prepared any style)	☐	☐	☐	☐	☐
Fresh cheeses (e.g. fresh ricotta, cottage cheese, cream cheese)	☐	☐	☐	☐	☐
Aged cheeses (e.g. Parmesan, strong cheddar)	☐	☐	☐	☐	☐
Smoked cheeses	☐	☐	☐	☐	☐
Other cheeses (e.g. cheddar, marble, feta, havarti, mozzarella, Monterey jack, gouda, pecorino, Gloucester, Cheshire)	☐	☐	☐	☐	☐
Red meat (e.g. Beef, lamb, venison, bison) or Cold cuts (of all types)	☐	☐	☐	☐	☐
Smoked meat & pork	☐	☐	☐	☐	☐
Hot dogs, frankfurters, weiners	☐	☐	☐	☐	☐
Fresh fish	☐	☐	☐	☐	☐
Frozen fish	☐	☐	☐	☐	☐
Preserved fish (in oil, in salt, dried)	☐	☐	☐	☐	☐
Smoked fish	☐	☐	☐	☐	☐
Shellfish					
(i) Molluscs (cuttlefish, octopus, squid, mussels, clams, oyster, scallops, etc.)	☐	☐	☐	☐	☐
(ii) Crustaceans (prawns, scampi, lobster, shrimp, crab, etc.)	☐	☐	☐	☐	☐

2a*. Please indicate how often you generally consumed the following foods while you were a teenager (age 13-19 years).
(please select only one box per row).

	Never	Less than once/month	1-3 times/ month	Once/week	2-3 times/ week	More than 3 times/week
Cows' milk (liquid or reconstituted powdered)	☐	☐	☐	☐	☐	☐
Other type of milk (Specify: _____)	☐	☐	☐	☐	☐	☐
Yogurt	☐	☐	☐	☐	☐	☐
Eggs (prepared any style)	☐	☐	☐	☐	☐	☐
Fresh cheeses (e.g. fresh ricotta, cottage cheese, cream cheese)	☐	☐	☐	☐	☐	☐
Aged cheeses (e.g. Parmesan, strong cheddar)	☐	☐	☐	☐	☐	☐

Continues next page

	Never	Less than once/month	1-3 times/ month	Once/week	2-3 times/ week	More than 3 times/week
Smoked cheeses	☐	☐	☐	☐	☐	☐
Other cheeses (e.g. cheddar, marble, feta, havarti, mozzarella, Monterey jack, gouda, pecorino, Gloucester, Cheshire)	☐	☐	☐	☐	☐	☐
Red meat (e.g. Beef, lamb, venison, bison) or Cold cuts (of all types)	☐	☐	☐	☐	☐	☐
Smoked meat & pork	☐	☐	☐	☐	☐	☐
Hot dogs, frankfurters, weiners	☐	☐	☐	☐	☐	☐
Fresh fish	☐	☐	☐	☐	☐	☐
Frozen fish	☐	☐	☐	☐	☐	☐
Preserved fish (in oil, in salt, dried)	☐	☐	☐	☐	☐	☐
Smoked fish	☐	☐	☐	☐	☐	☐
Shellfish						
(i) Molluscs (cuttlefish, octopus, squid, mussels, clams, oyster, scallops, etc.)	☐	☐	☐	☐	☐	☐
(ii) Crustaceans (prawns, scampi, lobster, shrimp, crab, etc.)	☐	☐	☐	☐	☐	☐

2b*. We are particularly interested in how often you consumed the following specific types of fish as a teenager:

	Never	Less than once/month	1-3 times/ month	Once/week	2-3 times/ week	More than 3 times/week
Canned salmon	☐	☐	☐	☐	☐	☐
Fresh or frozen salmon (not including smoked)	☐	☐	☐	☐	☐	☐
Canned tuna	☐	☐	☐	☐	☐	☐
Fresh or frozen tuna	☐	☐	☐	☐	☐	☐
Trout, Carp	☐	☐	☐	☐	☐	☐
Halibut	☐	☐	☐	☐	☐	☐
Sardines, anchovies	☐	☐	☐	☐	☐	☐
Fresh or frozen mackerel	☐	☐	☐	☐	☐	☐
Cod	☐	☐	☐	☐	☐	☐
Herring	☐	☐	☐	☐	☐	☐
Grouper, swordfish	☐	☐	☐	☐	☐	☐
Flounder, sole, smelt	☐	☐	☐	☐	☐	☐
Pickerel, snapper, perch	☐	☐	☐	☐	☐	☐
Other: specify _____	☐	☐	☐	☐	☐	☐

3*. What type of water did you usually drink when you were a *teenager?* *(you can check more than one box per row)*

	No Consumption	For drinking	For cooking	To make coffee/tea/hot drinks	Don't remember
Well water, spring water	☐	☐	☐	☐	☐
Tap water	☐	☐	☐	☐	☐
Bottled water	☐	☐	☐	☐	☐
Don't know	☐	☐	☐	☐	☐

4*. How often did you use the following condiments and oils as a teenager (including as dressings, or sauces, and for cooking)?
(Please check only one box per row)

	Never	Less than once/ mth	1-3 times/ mth	Once/ week	2-3 times/ week	4-5 times/ week	More than 5 times/week
Butter	☐	☐	☐	☐	☐	☐	☐
Margarine	☐	☐	☐	☐	☐	☐	☐
Lard	☐	☐	☐	☐	☐	☐	☐
Mayonnaise	☐	☐	☐	☐	☐	☐	☐
Vegetable oil							
(i) Corn, sesame, walnut, sunflower, flaxseed, safflower oil	☐	☐	☐	☐	☐	☐	☐
(ii) Canola, peanut, olive, coconut, avocado, almond oil	☐	☐	☐	☐	☐	☐	☐
(iii) Other vegetable oils. Specify: _____	☐	☐	☐	☐	☐	☐	☐

5*. Did you take any of the following dietary supplements while you were a *teenager?*

	Yes	No	Don't know
Cod liver oil liquid	☐	☐	☐
Cod liver oil capsules	☐	☐	☐
Fish oil capsules	☐	☐	☐
Multivitamins	☐	☐	☐
Calcium	☐	☐	☐
Vitamin B12	☐	☐	☐
Vitamin C	☐	☐	☐
Vitamin D	☐	☐	☐

6*. Please report what type of milk you were given when you were a baby. *(You can select more than one box per column and line).*

	At Birth	From 1-3 mths	From 4-6 mths	From 7-9 mths	From 10 mths & older
Breast milk	☐	☐	☐	☐	☐
Artificial formula	☐	☐	☐	☐	☐
Other milk (e.g. cow, soy, etc.) Specify:_____	☐	☐	☐	☐	☐
Don't know	☐	☐	☐	☐	☐

SECTION 4: MEDICAL HISTORY

The following questions concern illnesses that you may have had when you were younger.

1*. Please indicate at what age you had the following illnesses or surgical interventions. To help you remember, think about which school grade you were in when you had the illness/surgery. Check all that apply.

	Didn't have	Don't know	Did have →	Age at diagnosis: 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
Tonsillectomy (tonsil removal)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Measles	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mumps	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rubella (German Measles)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken pox	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pneumonia (check as many times as applies)	☐	☐	☐	☐	☐	☐	☐	☐	☐

2a*. Have you had infectious mononucleosis (also called "mono" or "the kissing disease")?

Yes ☐ → go to question **#2b**

No ☐ Don't know ☐ → if no or don't know, skip to question **#4**

2b*. If yes, do you recall if you had a blood test done to verify the diagnosis?

Yes ☐ No ☐ Don't remember ☐

2c. At what age did you have mononucleosis?

0-5 yrs ☐ 6-10 yrs ☐ 11-15 yrs ☐ 16-20 yrs ☐ 21-25 yrs ☐ 26-30 yrs ☐

3a*.Do you remember in which month you were diagnosed with mono?

☐☐ 01-12 → If you know the month skip to question **#4.**

3b. If you don't remember the exact month, can you recall in which season you had mono?

Spring ☐ Summer ☐ Fall ☐ Winter ☐ Don't Remember ☐

4. Have you ever had a urinary tract infection (UTI)? If yes, please give your best estimate of the age(s) when it/they occurred.

No	Don't know	Yes →	Ages when UTI occurred. *(you can check more than one box in the same row)*: 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
☐	☐	☐	☐	☐	☐	☐	☐	☐

5*. Have you ever had a parasitic infection (eg. tenia or tapeworm, ossiuri, ascarides, giardia, cryptosporidium, etc.)? If yes, please give your best estimate of your age when it first occurred.

No	Don't know	Yes →	Age of first infection: 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
☐	☐	☐	☐	☐	☐	☐	☐	☐

6*. Do you have a history of allergy (such as conjunctivitis or red itchy watery eyes, rhinitis or runny nose, eczema, hives, asthma) to any of the following? If yes, please estimate the approximate age at which you experienced the first symptoms (i.e., when did the allergies begin?)

	No	Don't know	Yes	0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
Pollens	☐	☐	☐→	☐	☐	☐	☐	☐	☐
House dust	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Animal dander / fur	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Any food	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Other allergies Specify: _____	☐	☐	☐→	☐	☐	☐	☐	☐	☐

7. Has a doctor ever told you that you had any of the following disorders?

	No	Don't know	Yes	Age at first diagnosis		No	Don't know	Yes	Age at first diagnosis	
Systemic lupus erythematosus (Lupus)	☐	☐	☐→		yrs	Celiac disease	☐	☐	☐→		yrs
Rheumatoid arthritis	☐	☐	☐→		yrs	Psoriasis	☐	☐	☐→		yrs
Hypothyroidism	☐	☐	☐→		yrs	Leukemia	☐	☐	☐→		yrs
Hyperthyroidism	☐	☐	☐→		yrs	Hodgkin's lymphoma	☐	☐	☐→		yrs
Multiple sclerosis	☐	☐	☐→		yrs	Non Hodgkin's lymphoma	☐	☐	☐→		yrs
Optic neuritis	☐	☐	☐→		yrs	Melanoma skin cancer	☐	☐	☐→		yrs
Crohn's disease	☐	☐	☐→		yrs	Non-melanoma skin cancer	☐	☐	☐→		yrs
Ulcerative colitis	☐	☐	☐→		yrs	Kidney disorders	☐	☐	☐→		yrs
Type I diabetes mellitus (juvenile diabetes)	☐	☐	☐→		yrs	Other medical disorders, specify: _____	☐	☐	☐→		yrs

8. To your knowledge, does anyone in your family have a history of any of the following diseases?

	No	Father	Mother	Brother/Sister	Child	Don't know
Systemic lupus erythematosus (lupus)	☐	☐	☐	☐	☐	☐
Rheumatoid arthritis	☐	☐	☐	☐	☐	☐
Hypothyroidism	☐	☐	☐	☐	☐	☐
Hyperthyroidism	☐	☐	☐	☐	☐	☐
Multiple sclerosis	☐	☐	☐	☐	☐	☐
Optic neuritis	☐	☐	☐	☐	☐	☐
Crohn's disease	☐	☐	☐	☐	☐	☐
Ulcerative colitis	☐	☐	☐	☐	☐	☐
Type I diabetes mellitus (juvenile diabetes)	☐	☐	☐	☐	☐	☐
Celiac disease	☐	☐	☐	☐	☐	☐
Psoriasis	☐	☐	☐	☐	☐	☐
Leukemia	☐	☐	☐	☐	☐	☐
Hodgkin's lymphoma	☐	☐	☐	☐	☐	☐
Non Hodgkin's lymphoma	☐	☐	☐	☐	☐	☐

SECTION 5: SMOKING HABITS AND LIFESTYLE FACTORS

1. Have you ever been a regular smoker ("regular" = smoked one or more cigarettes per day for 6 months or longer)?

Yes ☐ No ☐→ if your answer is no skip to question **#5**

2. If yes, how many cigarettes per day on average did you smoke at the following ages?

	Never smoked	1-4 cig./day	5-10 cig./day	11-20 cig./day	21+ cig./day
11-15 yrs	☐	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐	☐

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3. At what age did you begin to smoke cigarettes daily? (Age)

4. How many years have you smoked in total? (Total years)

5. Did your mother smoke while she was pregnant with you?

No ☐ Don't know ☐ Yes ☐ → How many cigarettes per day did she smoke?

< 10 ☐ 10+ ☐

6. Did your father smoke inside the house when you were a child?

She was a non smoker ☐ No, she didn't ☐ Don't know ☐ Yes ☐ → How many cigarettes per day did he smoke inside the house?

< 10 ☐ 10+ ☐

7. Did your mother smoke inside the house when you were a child?

He was a non smoker ☐ No ☐ Don't know ☐ Yes ☐ → How many cigarettes per day did she smoke inside the house?

< 10 ☐ 10+ ☐

8. Did you live with anybody else who smoked inside the house before you were age 21?

No ☐ Yes ☐ →

Who?	How many cigarettes a day were smoked inside the house?	
Brother ☐	< 10 ☐	10+ ☐
Sister ☐	< 10 ☐	10+ ☐
Other ☐	< 10 ☐	10+ ☐

9. Did you live with anybody else who smoked inside the house when you were between the ages of 21-25?

No ☐ Yes ☐ → How many cigarettes per day were smoked inside the house?

< 10 ☐ 10+ ☐

10. Did you live with anybody else who smoked inside the house when you were between the ages of 26-30?

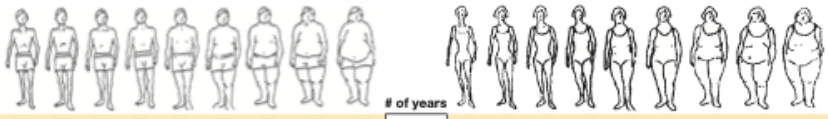
No ☐ Yes ☐ → How many cigarettes per day were smoked inside the house?

< 10 ☐ 10+ ☐

11. Have you ever worked in an environment where someone regularly smoked inside your workplace?

No ☐ Yes ☐

12*. MEN and WOMEN. What figure best depicts the shape of your body at the different ages. Also, please indicate approximately how long you were in this shape?



	# of years
At 5-years	
At 10-years	
At 15-years	
At 20-years	
At 25-years	
At 30-years	
Today	

13. What is your current weight? (Pounds) or (Kilograms)

14. What is your current height? (Feet & Inches) or (Centimeters)

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15. What was your level of physical activity per week when you were a teenager (between 13 and 19 years old)? (For example, light physical activities refer to activities that require light physical effort such as walking leisurely, stretching, vacuuming or light yard work. Vigorous physical activities refer to activities that take heavy physical effort such as jogging, running, stair machine, sports (e.g. tennis, basketball, soccer, etc.)).

	None	< once/week	1-2 times/week	3 or more times/week
Light physical activity (your heart beats slightly faster than normal)	☐	☐	☐	☐
Vigorous physical activity (your heart rate increases a lot)	☐	☐	☐	☐

SECTION 6: HORMONAL FACTORS WOMEN ONLY.
Men, please proceed to the last question (#14) on page 10.

1*. How old were you when you started menstruation? (started getting your period)? ☐☐ (Age)

2*. Are you pregnant now? Yes ☐ No ☐

3*. Have you ever been pregnant? Yes ☐ No ☐ → if no skip to question **#5.**

4*. If yes, please provide the following information on the outcome of each pregnancy and the year(s).

	1st pregnancy	2nd pregnancy	3rd pregnancy	4th pregnancy	5th pregnancy	6th pregnancy
Born alive	☐	☐	☐	☐	☐	☐
Breastfeeding for at least 1 month	☐	☐	☐	☐	☐	☐
Lost pregnancy (spontaneous or induced abortion, interuterine death, still born)	☐	☐	☐	☐	☐	☐
Lost at # weeks:	_____	_____	_____	_____	_____	_____
Year of outcome:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐

5*. Have you ever undergone hormonal treatment for infertility? Yes ☐ No ☐ → if no skip to question **#7.**

6*. If yes, please indicate the year(s) you received treatment and the number of cycles per year.

Year:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
No. of cycles/year:	☐☐	☐☐	☐☐	☐☐	☐☐

7*. Have you ever used a birth control pill (not the "mini-pill" that contains progesterone only, but the type that is taken for 3 weeks, followed by 1 week replacement with "sugar-pills"), hormonal patches, vaginal hormonal rings, or hormonal intrauterine devices (IUD)?
Yes ☐ No ☐ → if no skip to question **#10.**

8*. How old were you when you started using these contraceptives? ☐☐ Age

9*. For how long did you/have you used these contraceptives?

<1 year	1-3 years	4-5 years	6-9 years	10+ years
☐	☐	☐	☐	☐

10*. Have you ever suffered from hirsutism, that is, from an excess of coarse hair in areas of the body where it is not normally found (e.g. face, chest, back, abdomen)? Yes ☐ Don't know ☐ No ☐ → if no/don't know skip to last question **#14.**

11. If yes, have you ever been given hormonal therapies to treat this? Yes ☐ No ☐ → if no skip to last question **#14.**

12. At what age did you start these therapies? ☐☐ Age

13. For how long did you take these therapies?

<1 year	1-3 years	4-5 years	6-9 years	10+ years
☐	☐	☐	☐	☐

14. Lastly, we would like to know if someone else helped you fill out the questionnaire.

No ☐ Yes ☐ → Who?

Mother	Father	Sibling	Husband/Wife	Child
☐	☐	☐	☐	☐

End time of completed questionnaire: ☐☐ : ☐☐ **AM / PM (Circle one)**

Thank you for your participation!